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Cholinergic basal forebrain involvement in the acquisition of differential reinforcement of low rate responding tasks in rats

Sean Ryan Corley

CHOLINERGIC BASAL FOREBRAIN INVOLVEMENT IN THE ACQUISITION OF DIFFERENTIAL REINFORCEMENT OF LOW RATE RESPONDING TASKS IN RATS

A Thesis

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by

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ABSTRACT

The following experiments were designed to explore the potential role of the rat basal forebrain cholinergic system (BFCS) in the acquisition and performance of a standard (uncued) version and a cued version of the differential reinforcement of low rate responding (DRL) task. BFCS involvement in DRL behavior has not yet been systemically studied. In the standard DRL task, each trial consists of a designated interval of time which must elapse without the animal pressing a lever, with reinforcement following the first response after the required interval. If the animal responds before the interval is complete, it is not reinforced and the interval is reset. In the cued DRL task, animals must learn to respond to an external cue signaling the availability of reward, thus precluding the explicit need for timing behavior found in the uncued DRL task. The cued DRL task, therefore, provides a measure of response inhibition that should be insensitive to potential timing deficits associated with the uncued DRL task.

In the current experiments, it was hypothesized that 192 IgG-saporin lesions of the BFCS would disrupt DRL acquisition and performance in the uncued DRL task, but would not impair behavior in the cued version of the task.

Results from the current experiments suggest that BFCS lesions impair vigilance to external cues despite continued practice in the cued DRL, whereas continuous attention to internally produced cues recovers with extended practice in the uncued DRL.

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TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER ONE

ALZHEIMER'S DISEASE

Introduction

A minor decline in cognitive functioning is commonly associated with normal ageing (Muir, 1997). Extensive and progressive deterioration of cognitive capabilities to the point that social and occupational functions are significantly impaired, however, is abnormal and is a warning sign of major brain disturbances. Of the many brain disorders that severely diminish intellectual abilities in the elderly, dementia is most common. One study reports that 3.5-16.1 percent of the population aged 65 and older suffer from dementia, while the incidence of those afflicted under the age of 60 is minimal (Brookmeyer, Gray, & Kawas, 1998). Additionally, the prevalence of dementia increases exponentially with advancing age. For example, it has been reported that the prevalence ranges from 3.0 percent between 65 to 74 years to as much as 47.2 percent for those aged 85 and older (Evans et al., 1989; Wernicke & Reischies, 1994).

What may be more disturbing is that, in the United States, the annual incidence (i.e., the number of new cases reported in a year) of dementia was found to be at

over 5 percent for the population 85 years and older (Gao, Hendrie, Hall, & Hui, 1998). Corroboratively, Kukull et al. (2002) recently reported the incidence of new cases of dementia in the United States to be 14 new cases per 1000 person-years per year in individuals aged 65 years and older, rising to greater than 56 new cases per 1000 person-years per year in those aged 90 years or older. Of additional interest, women have been found to be slightly more prone to develop dementia as compared to men (Gao et al., 1998), perhaps because of the greater longevity of women. As the size of the elderly population continues to grow in modern societies, due in large part to the low mortality rates encountered in this age group over the last few decades, the incidence of dementia cases, especially in those aged 65 and older, should also inevitably rise above previous estimates (Katzman, 1986).

The most prevalent of the various types of dementia is Alzheimer's disease (AD). AD has been reported to account for approximately 50-70% of dementia cases (Terry & Katzman, 1983; Tomlinson, Blessed, & Roth, 1970). At present, an estimated 4.5 million people in the United States are afflicted with AD, according to recent census data (Hebert, Scherr, Bienias, Bennett, & Evans, 2003). This current estimation of Americans suffering from AD has

more than doubled since 1980 (Hebert et al., 2003). With the ever-growing population, the number of Americans with AD is expected to reach somewhere between 11.3 million to 16 million by the year 2050 (Hebert et al., 2003).

Although AD is occasionally observed in the younger population, the number of those afflicted in this age group is only minimal. Conversely, the greater occurrence of this brain disorder usually occurs in older aged individuals. For instance, Evans et al. (1989) found the percentage of people suffering from AD to be approximately 5 percent in those aged 65 to 74 years and close to 50 percent in those over 85. Moreover, the incidence of AD is highest among people in their 90s, although a decrease in the number of those afflicted may be observed for those who live past their ninth decade of life (Katzman, 1986).

In AD, cognitive functioning progressively deteriorates and death ultimately occurs, usually from accompanying medical complications, most notably bronchitis or pneumonia (Cummings & Cole, 2002). After prognosis of AD, the expected life span of an individual typically ranges between 7 to 10 years (Brookmeyer, Corrada, Curriero, & Kawas, 2002). Although cognitive decline is intimately connected with AD, behavioral disturbances such as depression, aggression, and wandering

have been known to develop over time as well (Francis, Palmer, Snape, & Wilcock, 1999). These incapacitating effects of AD paired with the accelerated growth of AD cases, especially among the elderly population, causes great concern to the public health care system, considering that institutional or full-time care will need to be provided to these individuals when they are eventually no longer able to care for themselves (Katzman, 1986). A way to circumvent these expected costs would be to develop an agreed upon way to diagnose and treat this disorder early, before it has a chance to produce its debilitating effects.

Over the years, significant advances have been made in the clinical diagnosis of AD. In the past, the clinical diagnosis of AD reportedly had an error rate of 10 to 50 percent (Garcia, Reding, & Blass, 1981), but in recent years, the diagnostic accuracy has improved to somewhere between 87 and 96 percent (Galasko et al., 1994; Gearing et al., 1995; Klatka, Schiffer, Powers, & Kazee, 1996; Kosunen et al., 1996). Neuropsychological tests, for example, have been claimed to be able to report mild learning and memory deficits in people who will later develop AD even before clinical symptoms are undeniably apparent (Linn et al., 1995). It should be noted,

however, that minor deficits in brain functioning also occur with normal ageing (Morrison & Hof, 1997). Therefore, it is not until AD progresses that performance differences become evident between normal elderly individuals and AD patients. Additionally, differentiating AD from other diseases that produce dementia has proven to be even more problematic (Dickson, 2001). This observed overlap of mental deficits between AD, other dementia diseases, and normal ageing populations, following peripheral diagnostic techniques, has made the task of definitively diagnosing AD in the clinical setting a difficult task. Until a universally accepted peripheral diagnostic marker is discovered for AD, the only currently agreed upon method to definitively diagnose AD is through microscopic examination of brain tissue at autopsy and/or biopsy (Mirra, Hart, & Terry 1993).

Neuropathology of Alzheimer's Disease

Preceding the microscopic histopathological examination of AD brain tissue, virtually all AD brains show some visible degree of cerebral atrophy (Dickson, 2001). The main targets of this irreversible form of brain atrophy are the hippocampus, and the frontal,

temporal, and parietal lobes (Ladner & Lee, 1998). Even though brain atrophy is clearly observable in the postmortem brains of AD patients, normal age-matched individuals also exhibit a variable degree of brain degeneration (Dickson, 2001; Perl, 2000). Therefore, an individual cannot be diagnosed with AD based solely on the presence of brain atrophy. Instead, a microscopic examination of brain tissue at autopsy and/or biopsy is conducted to search for the major microscopic neuropathological features of AD. The two cardinal microscopic neuropathological AD markers are the presence of intracellular neurofibrillary tangles (NFTs) and extracellular deposits of β-amyloid protein (Aβ) within senile plaques in the brain tissue (Katzman, 1986). These tangles and plaques are present throughout the cerebral cortex and the hippocampus.

Pioneering ultrastructural studies discovered that NFTs, located within the neuronal cell body, are irregular neurons composed of elongated bundles of abnormal filaments. These filamentous structures appear in pairs that are wound around each other in a helical manner, which gave rise to the term paired helical filaments (PHFs; Kidd, 1963; Wisniewski, Narang, & Terry, 1976). Later protein chemical characterization research revealed

that the primary component of NFTs is tau, a microtubule-associated phosphoprotein that forms part of the intracellular support system of neurons (Grundke-Iqbal et al., 1986; Kondo et al., 1988; Nukina & Ihara, 1986; Wischik et al., 1988). It is believed that PHFs accumulate in the neuronal cell bodies and extend into the dendrites of AD brains possibly because of tau that has been abnormally phosphorylated (Morishima-Kawashima & Ihara, 2002).

Senile plaques, the other classic neuropathological feature of AD, are complex structures that consist of Aβ protein deposits surrounded by abnormally shaped neuronal terminals (axons and dendrites) in the extracellular space of the brain tissue (Perl, 2000). Aβ protein is the by-product of a large protein, located within brain cells, called amyloid precursor protein (APP; Kang et al., 1987). The normal function of APP is still not fully understood, although it has been implicated in adhesion, neurite outgrowth, long-term potentiation, and neuronal migration (see Koo, 2002). In AD, APP is commonly cleaved to form a long protein, having 42 amino acids, called β-amyloid protein 42 (Aβ42) and it is this protein that tends to be deposited within the amyloid cores of senile plaques and impairs neuronal function (Prelli, Castano, Glenner, &

Frangione, 1988). Additionally, it is believed by some that the production, aggregation, and deposition of Aβ42 protein are responsible for the observed pathogenesis of AD (Younkin, 1998).

Another, but no less important, neuropathological feature of AD that accompanies the above mentioned accumulation of tangles and plaques is the loss of neurons and synapses (Terry et al., 1991). Yet, the way in which neuron and synapse loss relates to the formation of tangles and plaques in the AD brain is still not definitively known. Of importance, however, is that neuron and synapse loss results in a reduction of post-synaptic activity (Nitsch, 1996). In parallel, the loss of presynaptic neuronal structures leads to the reduction of neurotransmitter levels in the AD brain (Nitsch, 1996).

The deterioration of many neurotransmitter systems has already been identified in AD, including the cholinergic, serotonergic, glutamatergic, and peptidergic systems (Bowen et al., 1983; Francis, Sims, Procter, & Bowen, 1993; Palmer et al., 1987; Procter et al., 1988). The degeneration of the basal forebrain cholinergic system (BFCS), specifically the cortically-projecting neurons of the nucleus basalis of Meynert (NBMeynert) and the hippocampally-projecting neurons of the medial septum

(MS), have long been regarded as one of the earliest pathological features of AD (Nakano & Hirano, 1982; Whitehouse, Price, Clark, Coyle, & DeLong, 1981; Whitehouse et al., 1982). In conjunction with cell loss within the BFCS, especially in the NBMeynert, a concomitant loss of the BFCS primary neurotransmitter acetylcholine (ACh) has been reported in a number of earlier studies (Bowen, Smith, White, & Davison, 1976; Davies & Maloney, 1976; Perry, Perry, Blessed, & Tomlinson, 1977).

An important early finding, and the major neurochemical change exhibited in the postmortem brains of AD patients, was the report of a significant loss of choline acetyltransferase (ChAT), the synthesizing enzyme for ACh and a biochemical marker for cholinergic activity, in the cerebral cortex and hippocampus of AD brain tissue (Bowen et al., 1976; Davies & Maloney, 1976; Perry et al., 1977). Later studies additionally revealed significant decreases in high affinity choline uptake (Rylett, Ball, & Colhoun, 1983), ACh release (Nilsson, Nordberg, Hardy, Wester, & Winblad, 1986), and both nicotinic and muscarinic ACh receptor binding (Araujo, Lapchak, Robitaille, Gauthier, & Quirion, 1988; Aubert, et al., 1992; Nordberg, Alafuzoff, & Winblad, 1992; Perry et al.,

1995; Whitehouse et al., 1988) in postmortem AD brains compared to nondemented elderly controls.

These BFCS deficits severely compromise cognitive functioning (Collerton, 1986; DeKosky et al., 1992; Perry et al., 1978). For example, past research has revealed a strong correlation between changes in AD patients' premortem mental status scores and the reduction of ChAT activity or cholinergic neuronal loss from the NBMeynert (Perry et al., 1978; Wilcock, Esiri, Bowen, & Smith, 1982). These findings, together with the reports that centrally acting anticholinergic agents in normal humans produces cognitive deficits (Drachman, 1977; Drachman & Leavitt, 1974; Drachman & Sahakian, 1980) partly resembling those seen in AD led to the formulation of the cholinergic hypothesis of age-related memory loss and AD (Bartus, 2000; Bartus, Dean, Beer, & Lippa, 1982; Perry, 1988).

The cholinergic hypothesis of AD proposes that the ACh deficiency resulting from BFCS deterioration leads to the cognitive impairments associated with AD. Enhancement of cognitive functioning in normal humans after administration of cholinergic agonists (Davis et al., 1978; Sitaram, Weingartner, & Gillin, 1978) added further support to the cholinergic hypothesis of cognitive

impairment in AD. Based on these findings, pharmacological therapies that modify cholinergic neurotransmission have been developed to treat AD. Of the many types of drugs used to modify cholinergic neurotransmission, the cholinesterase inhibitors have proven to be most effective in the treatment of the cognitive impairments observed in AD (Gauthier, 2002). However, these therapeutic strategies have only met with limited success in alleviating the cognitive deficits of AD; typically these drugs only delay the progression of cognitive impairments in AD. Much research still needs to be performed in order to fully understand how changes in the central cholinergic system relate to AD pathology and ways to treat or prevent them from occurring.

Cognitive Impairments of Alzheimer's Disease

AD is characterized by a gradual onset and a slow progressive cognitive decline (Burns, Jacoby, & Levy, 1991). Consequently, it has been difficult to identify AD at an early stage considering that a minor decline in cognitive functioning is also associated with normal ageing. The dilemma, therefore, is that early assessment of AD is critical if future treatment opportunities are potentially going to be effective. Despite these

pitfalls, many experimental studies have investigated AD patients in more advanced stages of the disease. Among the numerous cognitive and behavioral disturbances demonstrated by these AD patients, learning and memory deficits as well as perseverative or intrusive behaviors are common. The following sections will review each of these positions below.

Memory Impairments

Past neuropsychological approaches have focused heavily on changes in explicit (conscious) memory, particularly episodic memory (the conscious recollection of previous personal experiences or events) in AD patients. Episodic memory has been traditionally assessed through free recall (i.e., the free reproduction of previously presented material, such as list-learning tests), cued recall (i.e., providing cues for retrieval of previously presented material), and recognition (e.g., recognizing pictures) tests. Furthermore, these tests have been used to measure different perceptual modalities, mainly verbal and visual performance in AD patients.

A consistent finding is that performance on free recall of previously presented word lists is poorer in AD patients compared to normal elderly controls (Eslinger & Damasio, 1986; Martin, Brouwers, Cox, & Fedio, 1985;

Spinnler, Della Sala, Bandera, & Baddeley, 1988). Similarly, the performance of AD patients on cued recall tasks is impaired compared to normal elderly controls. For example, it has been reported that the addition of semantic cues does not aid recall performance of AD patients (Bird & Luszcz, 1991; Bondi & Kaszniak, 1991; Chertkow & Bub, 1990; Monti et al., 1996; Russo & Spinnler, 1994), quite possibly due to deficient semantic encoding. In support of this perspective, it has been suggested that AD patients are unable to make semantic associations between related concepts (Sailor, Bramwell, & Griesing, 1998). This impaired ability to discriminate between related concepts therefore may contribute to poor performance on semantically cued recall tests.

In addition to poor performance on recall tasks, AD patients, as compared to normal elderly controls, are also impaired on recognition tasks (Abbenhuis, Raaijmakers, Raaijmakers, & Van Woerden, 1990; Deweer, Pillon, Michon, & Dubois, 1993; Eslinger & Damasio, 1986; Fleischman et al., 1996; Grosse, Wilson, & Fox, 1990; Heindel, Butters, & Salmon, 1988; Koivisto, Portin, & Rinne, 1996; Russo & Spinnler, 1994). For example, Eslinger and Damasio (1986) reported the inability of AD patients to recognize previously presented pictures of unfamiliar faces.

Similarly, others have found that AD patients are impaired in their ability to recognize pseudowords (Keane, Gabrieli, Growdon, & Corkin, 1994).

Given that AD patients are similarly impaired on free recall, cued recall, and recognition tests (classical measures of episodic memory) it has been proposed that AD patients suffer from a general episodic memory impairment (Spaan, Raaijmakers, & Jonker, 2003). In addition, regardless of the perceptual modality of the stimuli (e.g., verbal or visual) used in the episodic memory paradigms, performance is inevitably impaired in each situation (Greene, Baddeley, & Hodges, 1996). Others have concluded that because free recall performance and recognition performance are equally impaired in AD patients, then these individuals may be suffering from a learning deficit due to poor encoding rather than impaired retrieval (Greene et al., 1996).

Other studies have focused attention on semantic memory, the other major explicit memory subsystem, in the AD population. Semantic memory refers to facts and general knowledge, including the mental lexicon (vocabulary), accumulated over an individual's lifetime. Semantic memory, unlike episodic memory, is not learning-context dependent (Spaan et al., 2003). That is, it is

not crucial for an individual to remember the exact situation in which a particular item of knowledge was acquired. Typical methods used to examine semantic memory include verbal (or category) fluency, word identification, and (object) naming tasks. In particular, the verbal (or category) fluency task, which requires the subject to name as many exemplars as possible of a specific category (e.g., animals, fruits, or vegetables) within a given time limit, has frequently been used to assess semantic memory function of AD patients.

Several studies have reported that AD patients regularly exhibit impaired verbal fluency performance (Beatty, Testa, English, & Winn, 1997; Hodges, Salmon, & Butters, 1990; Hodges & Patterson, 1995; Mickanin, Grossman, Onishi, Auriacombe, & Clark, 1994; Monsch et al., 1992; Sailor et al., 1998). For example, AD patients, as compared to normal elderly controls, generally name fewer correct exemplars as well as name only the most common or frequent items of a particular category (Beatty et al., 1997; Martin & Fedio, 1983). Although semantic knowledge is clearly disrupted in AD patients, broader categorical information (e.g., naming the most common items in a category) is relatively spared in these individuals (Martin & Fedio, 1983). These

results taken together led to the conclusion that loss of knowledge in AD, rather than impaired retrieval, disrupts performance on the verbal fluency task (Monsch et al., 1994; Randolph, Braun, Goldberg, & Chase, 1993; Rosser & Hodges, 1994).

In addition, other studies have examined short-term or working memory performance of AD patients. Short-term memory is the memory of an event that just happened. The traditional measures of short-term memory are memory span (the number of elements one can recall immediately after presentation) tasks. The most common memory span tasks are the auditory/verbal span and visuospatial span tasks. Of interest, is that in the AD population these two memory span tasks have produced different results. On the one hand, no difference in performance was observed between early (or minimal) AD patients and normal elderly controls on the auditory/verbal span task (Carlesimo, Fadda, Lorusso, & Caltagirone, 1994; Hodges & Patterson, 1995; Morris, 1994; but see Orsini, Trojano, Chiacchio, & Grossi, 1988). On the other hand, others have reported impaired visuospatial span performance (i.e., smaller visuospatial span) in early stage AD patients, as compared to normal elderly controls (Carlesimo et al., 1994; Orsini et al., 1988; Spinnler et al., 1988; Trojano, Chiacchio,

De Luca, & Grossi, 1994). Overall, these results suggest that visuospatial working memory is sensitive to the effects of AD even at the earliest stages of the disease.

Like the conflicting results of short-term memory in AD patients, implicit memory tasks have also revealed inconsistencies in these subjects as well. Implicit memory is the nonconscious influence of past knowledge on some future task (e.g., skills, habits). Implicit memory is commonly examined through priming and procedural memory (or skill learning) paradigms. Priming, for example, is the enhancement of performance on information that one has previously processed nonconsciously. Priming effects may be tested by perceptual identification of words, free association, lexical decision, word stem completion, word fragment completion, and picture completion tasks. Procedural memory (or skill learning), on the other hand, is the knowledge of how to perform particular tasks (i.e., the acquisition of skills), which may be, for example, motor, verbal, or cognitive skills. Skill learning is a process that develops and improves gradually over time with repeated exposure (i.e., practice) to the desired skill to be acquired. Examples of tasks measuring skill learning are serial reaction time, mirror tracing, pursuit rotor, reading transformed script and maze learning.

Implicit memory as measured by perceptual (identification) priming effects is preserved in AD patients despite poor explicit memory of the stimuli used. For example, AD patients, as compared to normal elderly controls, are unimpaired on many perceptually based priming tasks, including perceptual identification of words (Abbenhuis et al., 1990; Gabrieli et al., 1994; Keane, Gabrieli, Fennema, Growdon, & Corkin, 1991; Koivisto et al., 1996; Meiran & Jelicic, 1995; Russo & Spinnler, 1994), perceptual identification of pseudowords (Keane et al., 1994), reading mirror words (Deweer et al., 1993, 1994; Grober, Ausubel, Sliwinski, & Gordon, 1992), and perceptual identification of incomplete pictures (Gabrieli et al., 1994). However, the priming effects on word stem completion tasks are not as conclusive for AD patients. Several studies have reported impaired performance of AD patients on word stem completion priming tasks (Bondi & Kaszniak, 1991; Butters, Heindel, & Salmon, 1990; Carlesimo, Fadda, Marfia, & Caltagirone, 1995; Gabrieli et al., 1994; Keane et al., 1991, 1994; Meiran & Jelicic, 1995; Salmon, Shimamura, Butters, & Smith, 1988; Shimamura, Salmon, Squire, & Butters, 1987), while others have demonstrated intact performance (Grosse et al., 1990; Fleischman et al., 1996). Fleischman et al. (1996)

suggest that the differences observed in intact and impaired word stem completion priming AD studies may be due to the extent of brain deterioration in the AD patients tested in those studies.

Unlike the inconsistent performance of AD patients on priming tasks, AD patients display relatively intact procedural memory (or skill learning). For example, AD patients, as compared to normal elderly controls, demonstrate normal improvement over trials on perceptual-motor learning tasks (e.g., pursuit motor, mirror tracing, serial reaction time, and maze learning) and are able to transfer this implicit knowledge to similar novel trials, without explicit knowledge of doing so (Butters et al., 1990; Eslinger & Damasio, 1986; Gabrieli, Corkin, Mickel, & Growdon, 1993; Grosse, Wilson, & Fox, 1991; Heindel et al., 1988; Heindel, Salmon, Shults, Walicke, & Butters, 1989; Knopman, 1991; Knopman & Nissen, 1987). In addition to motor skill learning tasks, AD patients are able to perform proficiently on verbal-perceptual learning tasks. Many studies have found that AD patients are able to learn and perform as well as normal elderly controls on the mirror reading task, despite poor explicit recognition of the stimuli being used (Deweer et al., 1993, 1994). It should be noted, that one study did report that AD

patients demonstrate impaired learning of the mirror reading task (Grober et al., 1992). The impaired performance of AD patients in the Grober et al. (1992) study was interpreted to suggest that AD patients suffer from deficient abstract reasoning that denies the ability to transform rotated text. In addition, Grober et al. (1992) used AD patients that were significantly older than the normal elderly controls (83.4 and 76.9, respectively), whereas the other studies used subjects that were in their 70s.

Perseveration

Among the many behavioral manifestations associated with the cognitive deterioration in AD, intrusive and perseverative behaviors, both instances of response disinhibition (the inability to withhold inappropriate responses), are often exhibited by patients suffering from AD (Bayles, Tomoeda, McKnight, Helm-Estabrooks, & Hawley, 2004; Caccappolo-van Vliet, Miozzo, Marder, & Stern, 2003; Fuld, Katzman, Davies, & Terry, 1982; Lamar et al., 1997; Loewenstein et al., 1989, 1991; Neils-Strunjas, Shuren, Roeltgen, & Brown, 1998; Rouleau, Salmon, Butters, Kennedy, & McGuire, 1992; Schram, Rubert, & Loewenstein, 1995; Sebastian, Menor, & Elosua, 2001). Perseveration is the inappropriate repetition of part or all of a previous

response. Intrusion, alternatively, is sometimes referred to as a type of delayed (recurrent) perseveration. It is worth noting that the responses that are repeated are, in themselves, not inappropriate - it is the failure to inhibit the response once it is completed that appears to be the problem in AD. Some have suggested that perseveration reflects a problem of "central executive" function (high level cognition), where there may be a problem updating the contents of working memory (Sebastian et al., 2001).

Sandson and Albert (1984) have classified three types of perseveration: continuous, recurrent, and stuck-in-set. A continuous perseveration is the inappropriate repetition of a behavior without an intervening response. A recurrent perseveration, on the other hand, is the inappropriate repetition of a previous response after intervening stimuli or responses, or both. The term intrusion has been interchangeably used instead of recurrent perseveration (see Fuld et al., 1982). Finally, stuck-in-set perseveration is the inability to cognitively switch to another category or task. Typical perseverative behaviors exhibited by AD patients are the repetition of the same ideas, movements, words, or thoughts. Neils-Strunjas et al. (1998), for example, indicated that AD

patients sometimes make continuous perseverations of single letters when writing (e.g., the word "under" is spelled as "undder"), a term referred to as letter perseveration. Caccappolo-van Vliet et al. (2003) also reported repetition of single letters in the AD population on writing tasks, especially on high frequency letters.

Another task that has been used to assess perseverations in AD patients is the "Clock Drawing Test", which, as the name states, requires the test taker to draw the face of a clock based on a picture representation provided by the experimenter. Rouleau et al. (1992) reported that patients in the advanced stages of AD tend to make continuous written perseverations of the clock numbers (e.g., 1, 2, and 3 O'clock may be drawn correctly, but the number 3 may be repeated where the 4, 5, and 6 O'clock positions are located).

Another form of perseveration, verbal perseveration, is observed in AD patients tested on verbal tasks. Verbal perseveration is classified as the oral repetition of a phoneme, syllable, word, or phrase. Fuld et al. (1982) were among the first to report the frequent occurrence of verbal intrusions, a type of recurrent verbal perseveration, among AD patients. Recently, Bayles et al. (2004) reported that AD patients as compared to aged

matched normal elders produced significantly more verbal perseverations. However, specific type of verbal perseveration, continuous, recurrent, or stuck-in-set, was not distinguished in this study. Instead, all three types of perseverations were recorded as a single category.

Other AD studies involving verbal tasks have differentiated a special form of response disinhibition in AD patients, defined as semantic intrusion or semantic perseveration (Lamar et al., 1997; Loewenstein et al., 1989, 1991; Schram et al., 1995). Although it was reported that general test intrusions or recurrent perseverations were not found to be specific to AD in early studies by Loewenstein and colleagues (1989, 1991), intrusions or perseverations of the more specific semantic form occurred with greater frequency in patients with AD relative to other disorders. Schram et al. (1995) compared intrusive or perseverative error responses of AD patients to random responses (called "guesses") of normal elderly controls to further distinguish if the errors verbalized were reflective of true semantic intrusions or perseverations, or were mere guesses. The results revealed that the intrusive or perseverative error responses of AD patients were different in both type and frequency as compared to the guesses of normals. Based on

the results, Schram and colleagues (1995) concluded that intrusive or perseverative error responses of AD patients are more reflective of true semantic intrusions or perseverations rather than random responding. Similarly, Lamar et al. (1997) found that the perseverations emitted by AD patients correlated most highly with the semantic form as compared to normal elderly controls, yet there was no difference between AD and subcortical ischemic vascular dementia (IVD) patients on this semantic perseveration subscale. Although semantic perseverations were not found to be specific to AD in this study, IVD and AD patients could be distinguished from one another in that perseverations produced by IVD patients correlated highest on motor and frontal systems tests, which were not found to be highly correlated in AD patients.

CHAPTER TWO

ANIMAL MODELS OF ALZHEIMER'S DISEASE

The BFCS has been argued to play a significant role in such cognitive processes as learning, memory, and attention (Everitt & Robbins, 1997). The rat BFCS, including the cortically-projecting nucleus basalis magnocellularis (NBM) and the hippocampally-projecting medial septum/vertical limb of the diagonal band of Broca (MS/VDB), closely parallels and is in many respects analogous to the NBMeynert and medial septum/diagonal band of Broca nuclei in humans (Fibiger, 1982), that are damaged in AD. Recall that there is a significant loss of basal forebrain cholinergic neurons in AD patient brains, especially the cortically-projecting neurons of the NBMeynert (Nakano, & Hirano, 1982; Whitehouse et al., 1981, 1982). The result of this basal forebrain cholinergic neuronal loss in AD patients is decreased cortical release of ACh (Nilsson et al., 1986), the primary neurotransmitter of the BFCS, and a corresponding decline in cognitive functioning (Collerton, 1986; DeKosky et al., 1992; Perry et al., 1978). To more closely study and understand the cognitive deficits of AD associated with this specific type of neuronal loss, rodent and

primate animal models involving lesions of the BFCS with subsequent analyses of changes in brain and behavior have been developed. Early work involved testing of various behavioral paradigms to assess cognitive function following excitotoxic (e.g., ibotenic acid or quisqualic acid) lesions of the BFCS, in particular the neurons of the NBM (for review see Dunnett, Everitt, & Robbins, 1991). A major downfall of these early lesion techniques of the basal forebrain was the lack of specificity for cholinergic neurons. This lack of selectivity caused difficulty in attributing the behavioral impairments observed to damage of cholinergic neurons as opposed to damage of surrounding noncholinergic neurons or fibers, or to the combined effect of cholinergic and noncholinergic damage (see Dunnett, Whishaw, Jones, & Bunch, 1987; Riekkinen, Riekkinen, & Riekkinen, 1991). Moreover, these early studies involving excitotoxic lesions of the basal forebrain called into question the contributions of the BFCS in cognitive function because the largest reductions in cortical cholinergic markers failed to result in the most profound behavioral deficits (Dunnett et al., 1987, 1991). The development of the more selective cholinergic immunotoxin 192 IgG-saporin (SAP; Wiley, Oeltmann, & Lappi, 1991), which selectively targets and destroys

cholinergic neurons of the basal forebrain in rats while sparing nearby noncholinergic neurons and fibers (Torres et al., 1994), offers a distinct advantage over alternative methods in understanding the specific cognitive contributions of the BFCS. This specificity is achieved by selective targeting of the p75 low-affinity neurotrophin (nerve growth factor) receptors located on the cholinergic neurons of the basal forebrain in rats (Heckers et al., 1994; Wiley, Berbos, Deckworth, Johnson, & Lappi, 1995). SAP binds to the p75 receptor and is internalized, whereupon the saporin moiety disrupts intracellular protein synthesis, ultimately leading to cell death (Wiley et al., 1991).

The increased experimental use of this selective immunotoxin further isolated and revealed the more specific behavioral role of the basal forebrain following loss of cholinergic neurons. Many behavioral impairments observed following less selective damage (e.g., excitotoxic lesions) to the basal forebrain, previously attributed to cholinergic dysfunction, are not replicated with the more selective cholinergic damage caused by SAP (see Wenk, 1997; Wrenn & Wiley, 1998). Briefly, some of the common behavioral tasks revealing behavioral deficits following less selective excitotoxic lesions of the basal

forebrain, in particular the NBM, are spatial learning (Kesner, Berman, & Tardif, 1992; Kwo-On-Yuen, Mandel, Chen, & Thal, 1990; Mandel, Gage, & Thal, 1989), avoidance learning (Dunnett et al., 1987; Flicker, Dean, Watkins, Fisher, & Bartus, 1983; Hepler, Wenk, Cribbs, Olton, & Coyle, 1985), and working memory (Biggan, Beninger, Cockhill, Jhamandas, & Boegman, 1991; Wozniak, Stewart, Finger, Olney, & Cozzarri, 1989). Whereas the absence of behavioral impairments in spatial learning (Baxter, Bucci, Gorman, Wiley, & Gallagher, 1995; Baxter et al., 1996; Dornan et al., 1997; Torres et al., 1994; Wenk, Stoehr, Quintana, Mobley, & Wiley, 1994), avoidance learning (Wenk et al., 1994; but see Zhang, Berbos, Wrenn, & Wiley, 1996), and working memory tasks (Baxter et al., 1995; Curzon, Bannon, & Decker, 1999) has been regularly demonstrated with more selective basal forebrain cholinergic damage inflicted from SAP lesions.

Dissociating whether the NBM and MS/VDB have similar or different roles in terms of cognitive functions has proven to be a challenging task. Initial studies using excitotoxic lesions to replicate cholinergic hypofunction, were mixed, reporting both qualitatively similar (Hepler, Olton, et al., 1958; Hepler, Wenk, et al., 1985) and different (Hagan, Salamone, Simpson, Iversen, & Morris,

1988) roles of these basal forebrain structures. In particular, the use of spatial learning tasks to compare the function of these two areas is a main reason for these conflicting viewpoints. Hagan et al. (1998) reported that excitotoxic ibotenic acid lesions of the MS/VDB in rats impaired spatial leaning and memory in a water maze task, while no deficit was observed on this task following NBM lesions. These results by Hagan and colleagues (1998) in addition to similar spatial studies involving nonselective lesions of the MS/VDB (Decker, Radek, Majchrzak, & Anderson, 1992; Kelsey & Vargas, 1993; McAlonan, Dawson, Wilkinson, Robbins, & Everitt, 1995), led researchers to suggest that the cholinergic innervation to the hippocampus, originating from the MS/VDB, plays a major role in spatial learning and memory. However, it should be noted that Whishaw and colleagues (1985) found impaired acquisition of spatial learning following excitotoxic ibotenic acid lesions of the NBM, therefore providing evidence that nonselective lesions of the NBM may be sufficient to disrupt spatial learning as well.

Combined basal forebrain (NBM + MS/VDB) excitotoxic lesion studies added further confusion to the functional role of these basal forebrain structures. For example, some studies reported that combined NBM + MS/VDB

excitotoxic lesions resulted in qualitatively similar behavioral deficits to excitotoxic lesions of either area alone (Hepler, Olton, et al., 1985; Hepler, Wenk, et al., 1985), while others have reported that the behavioral effects of combined lesions are additive to the effects of single lesions alone (Arendt et al., 1989). Additionally, Arendt et al. (1989) suggested that the BFCS works as a unitary structure with no functional difference between the individual components (i.e., the NBM and the MS/VDB) based on their observations that deficits on a radial maze task where more pronounced in rats with combined lesions as compared to rats with single lesions.

The advent of the cholinergically selective immunotoxin SAP, however, has allowed a growing consensus regarding the functional role of the components of the cholinergic basal forebrain (i.e., the NBM and the MS/VDB) in various types of behavioral tasks. In the following sections, studies involving SAP lesions of the NBM, the MS/VDB, or combined NBM + MS/VDB lesions in rats will be reviewed.

Nucleus Basalis Magnocellularis

The increased experimental use of the selective immunotoxin SAP has helped clarify the functional role of

the NBM following loss of cholinergic neurons. Several researchers propose that the NBM and its cortical cholinergic projections are directly involved in such cognitive processes as attention, cognitive flexibility, strategy switching, and configural association learning. The following sections will provide an overview of each of these perspectives.

Attention Perspective

There is a growing body of literature reporting impairments in tests of attention following intra-NBM SAP lesions in rats (Baxter et al., 1995; Chiba, Bucci, Holland, & Gallagher, 1995; Lehmann, Grottick, Cassel, & Higgins, 2003; McGaughy, Dalley, Morrison, Everitt, & Robbins, 2002; McGaughy, Decker, & Sarter, 1999; McGaughy, Kaiser, & Sarter, 1996; Risbrough, Bontempi, & Menzaghi, 2002; Turchi & Sarter, 1997). For example, the attention taxing tasks that have been shown to be sensitive to SAP lesions of the NBM include divided attention paradigms (Turchi & Sarter, 1997), operant measures of sustained attention or vigilance (McGaughy et al., 1996, 1999), visuospatial attention in five-choice serial reaction time (5CSRT) tasks (Chudasama, Dalley, Nathwani, Bouger, & Robbins, 2004; Lehmann et al., 2003; McGaughy et al., 2002; Risbrough et al., 2002), and incremental attention

in Pavlovian conditioning paradigms (Baxter et al., 1995; Chiba et al., 1995).

McGaughy et al. (1996) studied the performance of rats with SAP lesions of the NBM on an attention demanding behavioral vigilance task. This task involves signal versus nonsignal discrimination and requires the ability to detect visual signals of varying length. Further, the task provides a measure of four distinct response types: hits, misses, correct rejections, and false alarms. Preoperative (i.e., baseline) training of the behavioral vigilance task required rats to discriminate between signals (1-s illumination of center panel light) and nonsignals (trials with no illumination of center panel light) as well as requiring the animal to detect signals of variable length. A stable performance criterion of 70% correct responding on the longest signal and nonsignal trials and an omission rate of less than 40% on all trials had to be demonstrated before rats underwent surgery. Upon recovery from SAP lesions of the NBM, rats were returned to the behavioral vigilance task to characterize any change in postoperative performance as compared to preoperative performance. Postoperatively, NBM lesioned rats' ability to correctly reject nonsignals was not affected, but the ability to detect signals (hits) was

impaired. Additionally, NBM lesioned rats never recovered to preoperative performance on signal detection over the course of 180 sessions of postoperative testing. The finding that NBM cell loss results in an impairment in signal detection was viewed as providing support for the position that the cholinergic NBM plays a critical role in the detection of significant stimuli in situations requiring sustained attention or vigilance. In addition, the finding that signal detection is impaired but nonsignal rejection is unaffected following SAP lesions of the NBM was viewed as reflecting a possible dissociation between the cognitive processes associated with these two forms of responding.

Turchi and Sarter (1997) further explored the role of the NBM on attention processing in rats tested on a crossmodal divided attention task. This task involves conditional discriminations of both visual and auditory cues. Blocks of testing consisting of only visual or only auditory stimuli (i.e., unimodal testing) were identified as the modality certainty condition, whereas random mixed presentations of visual and auditory stimuli (i.e., bimodal testing) was identified as the modality uncertainty condition and was argued to tax attentional processing capacity. Stable preoperative baseline

performance of at least 70% correct responses on the unimodal conditions and no less than 57% correct responses on any one stimulus during uni- or bimodal testing was required before rats underwent surgery. Postoperative testing consisted of rats being reintroduced to the crossmodal divided attention task. The response latencies of NBM lesioned rats under the bimodal condition were longer as compared to their performance during unimodal conditions. Conversely, there was no difference of response latencies in the sham-lesioned group when unimodal and bimodal latencies were compared. In addition, it was found that a speed-accuracy tradeoff occurred with lesioned-induced damage to the NBM. That is, response accuracy increased at the cost of longer response latencies. This impairment of increased response latency during the bimodal blocks of trials (i.e., the attention-demanding condition) following SAP lesions of the NBM was viewed as support for the argument that the cholinergic NBM is involved in the regulation of attentional processing capacity.

Additionally, Chiba et al. (1995) investigated the ability of rats with SAP lesions of the NBM to increase or decrease attention to conditioned stimuli. Prior to behavioral procedures, SAP lesions of the NBM were

performed. To assess increased attentional processing of conditional stimuli, rats were tested on a serial conditioning task where the predictive value of conditioned stimuli was shifted at mid-training relative to initial training conditions. Comparisons were made between controls and lesioned rats on the strength of conditioned approach to a food cup in response to conditioned stimuli presentations predicting a food unconditioned stimulus. The amount of conditioning responding (i.e., food cup responding) was lower in the NBM lesioned group as compared to controls when the predictive value of the conditioned stimuli was adjusted during the course of training. When the predictive value of the conditioned stimuli was held constant, there were no differences between groups. The results were interpreted to suggest that the cholinergic NBM is critically involved in the ability to increase attention to sensory cues when the predictive value of the conditioned stimuli is modified.

In the same study, a different group of rats with SAP lesions of the NBM were tested on a latent inhibition task designed to assess any disruption of decreased attention to preexposed conditioned stimuli prior to standard classical conditioning. Latent inhibition occurs with

extensive nonreinforced preexposure to a conditioned stimulus prior to conditioning of that stimulus. The result is a retardation in conditioned responding to this preexposed conditioned stimulus during the acquisition phase of conditioning testing. The results revealed no differences on the latent inhibition task between control and lesioned rats; both groups showed the transient disruption of conditioned response acquisition typical of latent inhibition procedures. The failure to disrupt latent inhibition performance in the NBM SAP lesioned group showed that decreases in attention to conditioned stimuli are not critically influenced by cholinergic innervation of the cortex. Overall, cholinergic lesions of the NBM were found to disrupt incremental, but not decremental attentional processing in conditioning paradigms.

McGaughy et al. (2002) utilized the 5CSRT task to investigate the role of the NBM on visuospatial attention following intra-NBM injection of different doses of SAP in rats. The 5CSRT task required rats to respond to a brief visual stimulus (light) presented in one of five possible locations (square nose poke holes) in a completely randomized sequence. A nose poke response in the square hole where the light stimulus had been briefly presented

reflects correct responding, whereas incorrect responding occurs when a nose poke response is made to one of the four alternative square holes that were not illuminated. The following behavioral measures were used to assess performance on this task: accuracy (correct responses/correct + incorrect responses), omissions (failure to respond after stimulus presentation), premature responses (responses made after initiation of a trial but before light stimulus presentation), perseverative responses (additional responses after a correct response), and response speed (correct response latencies and magazine latencies). Once stable preoperative behavioral performance was reached, defined as greater than 80% accuracy and less than 25% omissions, surgeries were performed. One group of rats received a high dose of SAP infused into the NBM (HIGH), another group received a lower dose (LOW), and a control group received sham operations.

After recovery, rats were returned to the same standard 5CSRT task that they were preoperatively trained on, followed by testing on modified versions of the task designed to assess the potential effects of changes in attentional demands. Results revealed that extensive lesions of the NBM (SAP HIGH) impaired performance on the

5CSRT task. As compared to controls, the SAP HIGH group demonstrated deficits in correct response latencies, decreases in response accuracy, and increases in omissions, perseverative responses, and premature responses. On the other hand, the results indicated that the SAP LOW group exhibited fewer and weaker behavioral deficits. The SAP LOW group as compared to controls had a subtle postoperative decrease in response accuracy as well as a transient increase in premature responses, which was contingent on specific task parameters. Specifically, this accuracy deficit was most evident when sustained attentional demands were elevated by increasing event rate (i.e., lowering the inter-trial interval) and time on task.

In the same study, McGaughy et al. (2002) found that both greater loss of cholinergic NBM cells caused by SAP lesions and the resulting decreases in ACh efflux in the medial prefrontal cortex (measured via microdialysis) correlated with greater impairments on the 5CSRT task. Overall, the results provide further support for the argument that the cholinergic NBM is involved in the acquisition and performance of attention taxing tasks. Lesions created with lower doses of SAP demonstrated that even small reductions in cholinergic activity could cause

sustained attention deficits when attentional load is increased (see also Chudasama et al., 2004; Lehmann et al., 2003; Risbrough et al., 2002).

Cognitive Flexibility/Strategy Switching Perspective

In addition to a disruption of attention following cholinergic depletion of the NBM, recent studies have argued that SAP lesions of the NBM disrupt complex cognitive functions, which include cognitive flexibility and strategy switching behavior (Bailey, Rudisill, Hoof, & Loving, 2003; Butt & Bowman, 2002; Butt et al., 2003). For example, Bailey et al. (2003) examined the involvement of the cholinergic NBM on learning set (LS) performance in rats with SAP lesions. To assess LS formation following NBM surgeries, rats were tested on two learning set tasks: an olfactory discrimination learning set (ODLS) task and an olfactory discrimination reversal learning set (DRLS) task. The ODLS task used in this study employed a succession of two different odor-unique simple discrimination problems, five consecutive trials per problem, a day. Correct responses involved choosing an odor that was different from the remainder of the set. An example of LS formation on this ODLS task would be if chance performance occurs on the first trial of a new

odor-unique problem but above chance performance is observed on trial two of the same problem. Percentage of correct responses on the ODLS problems were significantly higher than chance on trials 2-5 in the control group, while NBM lesioned rats performed at greater than chance levels only on trial 5. In addition, greater than chance trial 2 performance on the ODLS task was acquired earlier in control rats than in NBM lesioned rats.

Following ODLS testing rats were shifted to the DRLS task to assess transfer of learning set formation on to a different LS task. The DRLS task required rats to initially make a simple olfactory discrimination between two different odor stimuli (banana and mint). Responding to the banana odor stimulus was initially reinforced while responding to the mint odor stimulus was not reinforced. Once the rats responded to the correct banana odor stimulus at a criterion of 80% or better for two consecutive blocks of testing trials, the reinforcement contingency was reversed (i.e., the correct odor was now the mint odor and the incorrect odor was now the banana odor). Upon reaching criteria again, rats underwent 14 additional reversals with the same two odor stimuli. Increasing performance on discrimination reversal tasks across extended testing is argued to be evidence of LS

formation in this particular paradigm. Bailey and colleagues found no difference in trials to criterion between the control and NBM lesioned group. Overall, the results were viewed as indicating that the cholinergic NBM is involved in the acquisition of LS formation as evidenced by the early, impaired performance of the NBM SAP lesioned group as compared to the performance of controls on the ODLS task. However, behavioral recovery of the NBM lesioned rats by the end of ODLS testing and lack of impairment after being transferred to a novel LS task demonstrated that cholinergic depletion of the NBM does not prevent eventual LS formation.

Configural Association Learning Perspective

Adopting a more traditionally associative learning perspective, rather than the cognitive perspective of the attention and LS arguments, Butt and colleagues (Butt & Bowman, 2002; Butt, Noble, Rogers, & Rea, 2002) have argued that the NBM is critically involved in complex or "configural" association learning, but not simple association learning. Their view that the NBM is involved in this form of complex learning has roots in configural association theory (see Pearce & Wilson, 1990; Rescorla, 1972, 1973; Sutherland & Rudy, 1989; Whitlow & Wagner, 1972). Configural association theory consists of two

types of associative learning: configural association learning and simple association learning. In configural association learning, reinforcement is contingent on the ability to solve learning problems where the solution depends on learning about the relationship between two or more stimulus events. Conversely, in simple association learning the solution to the problem is defined by a fixed and unambiguous contingency between a stimulus and its associated reinforcement outcome.

Previous research by Butt and colleagues has shown that SAP lesions of the NBM impair configural association learning but spare simple association learning in both negative patterning (Butt et al., 2002) and transverse patterning paradigms (Butt & Bowman, 2002). Butt et al. (2002) tested rats in either a simple discrimination task, to assess simple association learning, or a negative patterning task, to assess configural association learning, following bilateral SAP lesions of the NBM. Rats in the simple discrimination task were only reinforced if responses occurred during the presentation of a tone stimulus (T+) but not during light stimulus (L-) presentations. In the negative patterning task, a test of configural association learning, rats were reinforced for responding to either a tone (T+) or light (L+) presented

alone but responses made when the tone and light were presented together were not reinforced (LT-). The results indicated that SAP lesions of the NBM do not disrupt simple discrimination learning, but do impair acquisition of configural association learning in the negative patterning task. NBM lesioned rats made more LT- and inter-trial interval responses early in negative patterning testing but eventually performed as well as controls by the end of testing. The finding that configural association learning was impaired while simple association learning was spared following damage to the NBM was viewed as reflecting an inability to attend to multiple stimuli or events but not to attend to single stimuli. These findings were also interpreted as possibly reflecting an inability to switch between competing strategies to solve different problems.

Butt and Bowman (2002) similarly tested rats with SAP lesions of the NBM on a water-maze version of the transverse patterning task to provide further support to the notion that the cholinergic NBM is substantially involved in configural but not simple association learning. In the transverse patterning task, rats were trained to concurrently solve three different visual discrimination problems (Problem 1: A+ vs B-; Problem 2:

B+ vs C-; and Problem 3: C+ vs A-) during three successive phases (phase 1: A+ vs B-; phase 2: A+ vs B-, B+ vs C-; and phase 3: A+ vs B-, B+ vs C-, C+ vs A-). Problem 1 (A+ vs B-) and Problem 2 (B+ vs C-) were argued to be tests of simple discrimination, which could be solved by the use of simple associations, while Problem 3 (C+ vs A-), in conjunction with Problems 1 and 2, required the use of configural associations. The results supported the authors' argument that NBM lesions impair configural but spare simple association learning. The NBM lesion group was able to solve both Problems 1 and 2 (i.e., the simple association tasks) but showed a deficit in solving Problem 3 (i.e., the configural association task). Furthermore, during phase 2 testing (i.e., when Problem 2 was introduced and interspersed with Problem 1) NBM lesion rats, as compared to controls, performed poorly on the original Problem 1. This high level of performance on Problem 2 at the expense of Problem 1 was viewed as an inability to attend to multiple stimuli or switch between strategies to solve different problems. The findings that configural association learning and cognitive flexibility were both impaired after SAP lesions of the NBM was acknowledged by the authors as possibly reflecting an underlying impairment in attention.

Medial Septum/Vertical Diagonal Band

The increased experimental use of the selective immunotoxin SAP has additionally aided in clarifying the functional role of the MS/VDB following loss of cholinergic neurons. Several researchers propose that the MS/VDB and its hippocampal cholinergic projections are directly involved in such cognitive processes as spatial learning and memory, particularly spatial working memory, and attention. The following sections will review each of these perspectives below.

Spatial Learning and Memory Perspective

Early studies investigating the functional role of the cholinergic septohippocampal pathway suggested that the projections from the MS/VDB to the hippocampus play a key role in spatial learning and memory. For example, initial studies using nonselective excitotoxic lesions of the MS/VDB in rats revealed impairments on spatial learning and memory tasks (Hagan et al., 1988; Hepler, Olton, et al., 1985; Hepler, Wenk, et al., 1985). However, the more recent use of the highly selective cholinergic immunotoxin SAP has produced mixed results concerning the specific cholinergic involvement of the MS/VDB in spatial learning and memory.

There is a body of evidence which reports that SAP lesions of the MS/VDB in rats produce significant deficits in spatial learning and memory (Janis, Glasier, Fulop, & Stein, 1998; Johnson, Zambon, & Gibbs, 2002; Lamprea, Cardenas, Silveira, Morato, & Walsh, 2000; Lamprea, Cardenas, Silveira, Walsh, & Morato, 2003; Walsh, Herzog, Gandhi, Stackman, & Wiley, 1996). However, there are also a number of studies that report no substantial impairments on spatial learning and memory tasks following injections of SAP into the MS/VDB of rats (Bannon, Curzon, Gunther, & Decker, 1996; Baxter et al., 1995; Baxter & Gallagher, 1996; Berger-Sweeney et al., 1994; Cahill & Baxter, 2001; Chappell, McMahan, Chiba, & Gallagher, 1998; Dornan et al., 1997; Kirby & Rawlins, 2003; McMahan, Sobel, & Baxter, 1997; Pang & Nocera, 1999; Perry, Hodges, & Gray, 2001).

Baxter and Gallagher (1996), for example, have reported lack of impairment in water maze performance, a measure of spatial learning and memory, following infusions of SAP into the MS/VDB of aged rats. Dornan et al. (1997), as well as Torres et al. (1994), have also reported unimpaired water maze performance after MS infusions of SAP. Similarly, Perry et al. (2001) observed no deficits in spatial learning and memory on radial maze

and water maze tasks when rats were tested 5 or 11 months after site-specific SAP lesions of the MS. Berger-Sweeney et al. (1994) observed only a mild spatial learning impairment on the water maze after MS injections of SAP, which was evident only on the initial days of testing.

Spatial Working and Reference Memory. Baxter et al. (1995) demonstrated that intraparenchymal MS/VDB injections of SAP in rats do not impair acquisition of spatial memory in the water maze but did reveal a delay-independent impairment on a spatial working memory component of the task. These findings suggests that the MS/VDB may be involved in spatial working memory. However, the spatial working memory impairment reported by Baxter et al. (1995), has not been a consistent finding in the literature. For example, McMahan et al. (1997) reported no impairment in spatial working memory on a delayed non-matching to position (DNMTP) task following intra-MS/VDB SAP infusions in rats. Chappell et al. (1998) have reported a comparable lack of spatial working memory deficit after MS/VDB SAP lesions on the standard version of the radial maze. Moreover, increasing the delay between responses in the Chappell et al. (1998) study had no effect on the MS/VDB SAP lesioned group. Similarly, Kirby and Rawlins (2003) found no impairment in

spatial working memory on a preoperatively trained T-maze alternation task following intraseptal infusions of SAP. Pang and Nocera (1999) also demonstrated a lack of impairment in a T-maze alternation task following MS/VDB SAP infusions.

However, there are also a broad number of studies that report significant deficits in both spatial learning and working memory following injections of SAP into the MS/VDB. Among the behavioral impairments reported are impaired spontaneous alternation (Chang & Gold, 2004), impaired spatial strategy selection (Janis et al., 1998), impaired delayed matching to position (DMTP) in the T-maze (Johnson et al., 2002), impaired spatial working memory in the radial arm maze (RAM; Shen, Barnes, Wenk, & McNaughton, 1996; Walsh et al., 1996), and impaired spatial working memory in an operant DNMTP task (Torres et al., 1994).

Shen et al. (1996) tested rats with SAP lesions of the MS on two RAM tasks to assess both spatial working and reference memory performance. Rats were first trained on a spatial working memory task composed of pre-delay, delay, and post-delay stages. During pre-delay testing, rats were given free access to only four (randomly assigned daily) of the eight baited arms. Upon correctly

choosing the fourth arm, a varying delay (0, 1, 2 min) was enforced. After the delay, rats were returned to the maze with free access to all eight arms. During both pre-delay and post-delay stages, correct responses were recorded when the rats chose a baited arm that had not been previously explored, whereas spatial working memory errors were recorded when a rat re-entered an arm. Following stable performance (i.e., minimal re-entries across test days), SAP lesions of the MS were then performed and, upon recovery, rats were reintroduced to this spatial working memory task. Lesioned rats were found to make more spatial working memory errors (i.e., re-entering arms previously explored) during both pre-delay and post-delay stages as compared to control performance. The results demonstrate a delay-independent impairment in spatial working memory on the RAM in the SAP group. Although there was also a delay-dependent effect on performance across all animals, there was no difference between groups in the magnitude of this effect (i.e., the increase in delay did not differentially affect performance in the lesion and control groups).

Upon completion of the spatial working memory task, control and lesioned rats were shifted to a spatial reference memory task. The spatial reference memory task

allowed rats free access to all eight arms but two of the arms (at the same daily position) were never baited. Because the parameters did not change within or across trials, this task is said to test spatial reference memory (as opposed to spatial working memory). SAP lesions of the MS failed to impair acquisition of this task. The results of the study were viewed as providing support for the argument that the cholinergic MS and its hippocampal projections are involved in acquisition of spatial working memory for places recently visited but not long-term retention of this information (i.e., spatial reference memory). Furthermore, cholinergic MS involvement was not necessary for acquisition or retention of spatial reference memory for nonreward locations. This report of no spatial reference memory deficit following intraseptal SAP lesions is consistent with previous studies involving the use of water maze tasks (Baxter et al., 1995; Berger-Sweeney et al., 1994).

Walsh et al. (1996) in a similar study to Shen et al. (1996) found that intraseptal injection of SAP produced a dose-dependent deficit on a variable DNMTP RAM task. Prior to surgery, rats were trained on a standard RAM task, which allows free access to all eight baited arms of the maze. Re-entry into a previously explored arm was

recorded as an error. MS SAP lesions and sham surgeries were performed following completion of standard RAM training. After recovery, rats were tested on a DNMTP RAM procedure. Procedures were similar to those used by Shen et al. (1996) with the critical distinction that different delay times were used. On the first phase of DNMTP RAM testing, rats were provided with a delay of 1 h between pre-delay and post-delay arm presentations. A variable-delay DNMTP RAM task was initiated after the final day of the 1 h delay DNMTP RAM phase. Parameters were the same as before except that during this phase of testing a variable delay (15 min, 1 h, 4 h, 8 h) was imposed between pre-delay and post-delay arm presentations. Correct choices were recorded when the rats chose a baited arm that had not been entered earlier, whereas spatial working memory errors were recorded when rats re-entered a previously explored arm. SAP lesioned rats emitted more errors and a fewer number of correct responses than controls. The results observed after infusion of different doses of SAP into the MS were viewed as reflecting a spatial working memory deficit in the DNMTP task, where the magnitude of the spatial working memory impairment was influenced by cognitive demands of the task.

Similar impairments have been observed on an operant version of the DNMTP task following intraseptal SAP lesions in rats (Torres et al., 1994). In this study, rats were preoperatively trained on an operant DNMTP task. The task required rats to alternate bar presses between two retractable response levers. Between bar presses a randomly selected delay interval (0, 2, 4, 8, 12, 16, or 24 s) was imposed. Once rats maintained stable performance on the operant DNMTP task, surgeries were performed. Following recovery, rats were returned to the DNMTP task and postoperative performance (i.e., percent correct) was assessed. Results demonstrated that performance in the septal lesioned group was unimpaired at short delays as compared to control performance. However, the septal lesioned group showed a marked deficit in the percentage of correct responses at the two longest delays, indicating a delay-dependent impairment. The delay-dependent change was viewed as reflecting a short-term or working memory deficit. These results offered support for the argument that the cholinergic septohippocampal pathway is involved in short-term or working memory.

To broaden the understanding of the spatial processes affected by SAP lesions of the MS/VDB, Janis et al. (1998) investigated the effects of intraseptal SAP lesions on

strategy selection in both the spatial RAM and Morris water maze (MWM) tasks. In a spatial learning and memory task rats can use either an allocentric (place) strategy (e.g., spatial or working memory strategy) or an egocentric (response) strategy (e.g., response pattern or nonspatial strategy). First, rats were trained to exclusively enter each of eight novel, baited arms in an 8-arm radial maze without re-entering the searched arms during the trial. A working memory error was recorded when the rat re-entered a previously searched arm. Once a criterion of eight correct choices out of the first ten entries of a trial across 5 successive days of testing was met, performance of each rat was scored as an index of allocentric (place) or egocentric (response) strategies. Based on this scoring system rats were then classified as using either an allocentric or egocentric strategy to solve the task and surgeries were performed. Following recovery, rats were returned to the RAM task until preoperative criterion was reached. The results reveal that rats that used an allocentric, spatial strategy to solve the task were impaired following SAP lesions of the MS/VDB. The percentage of sequence repetitions, an index used to assess egocentric strategy, significantly increased in the allocentric group after SAP lesions but

not in the allocentric control group. Additionally, the percentage of spatial acuity, an index used to assess allocentric strategy, was significantly reduced postoperatively in the allocentric lesioned but not the allocentric control group. The results were viewed as demonstrating that a switch in allocentric to egocentric strategy occurs after cholinergic lesions of the MS/VDB on a RAM task.

In a continuation of the Janis et al. (1998) study, rats were then shifted to a MWM task after completion of the RAM task. Initially, rats were trained to find a visible platform in the MWM. Several times during visible platform testing, rats were given trials where the platform was hidden (submerged) but still in the same location. On the final day of testing, a probe trial was given in which the visible platform was moved to a different location. Rats were measured on how efficiently they could locate both the visible and hidden platform on the task. Both lesioned and control rats could locate the visible platform equally well, however, the lesioned group had a mild deficit in learning to locate the hidden platform as compared to control performance. In addition, lesioned rats as compared to controls preferred to swim directly to the new location on the probe trial, rather

than swimming to the old location than new location. This result was interpreted as providing further support that the use of an allocentric strategy may be impaired following cholinergic lesions of the MS/VDB. Overall, the results were viewed as demonstrating that SAP lesions of the MS/VDB produce impairments in spatial strategies that are utilized in spatial learning tasks such as the RAM and MWM.

Conversely, Cahill and Baxter (2001) report that SAP lesions of the MS/VDB do not impair place-learning strategy or strategy switching (i.e., switching from place to response strategies) of a spatial discrimination task in a plus shaped maze. In fact, SAP lesioned rats, as compared to controls, were facilitated on the acquisition of the spatial learning task. The results were viewed as demonstrating that the cholinergic involvement of the MS/VDB does not play a critical role in acquiring or using a place strategy to learn and solve a spatial discrimination task.

Past research has demonstrated that rats possess an innate tendency to spontaneously alternate between choosing accessible locations in a T-shaped or plus-shaped maze when released from a common starting location in the maze (Douglas & Raphelson, 1966; Still, 1966). More

recently, Chang and Gold (2004) investigated any possible deficits on a spontaneous alternation task after SAP lesions of the MS/VDB in rats. Lesions of the MS/VDB, as well as implantation of microdialysis cannulaes into the hippocampus, which receives cholinergic projections from the MS, were performed prior to behavioral testing. Upon recovery, rats were tested for spontaneous alternation performance in a plus-shaped maze. Once rats were placed in the maze, testing began and rats were permitted to move about freely in the maze for the 12 min testing period. During the testing session, entry into four different arms across overlapping sets of five successive entries was recorded as a spontaneous alternation for each rat. The results demonstrated that the SAP lesioned group exhibited a lower mean percentage alternation score as compared to controls. Even though MS/VDB lesions resulted in near total losses of ChAT-positive neurons in the area, ACh release to the hippocampus was not completely diminished, but was reduced to approximately 40% of controls. Therefore, these results provide evidence of residual cholinergic input to the hippocampus following this MS/VDB lesion. The results that SAP lesions of the MS/VDB impair spontaneous alternation were interpreted as suggesting that the cholinergic projections of the MS/VDB are

involved in spatial learning and memory, where this impairment was hypothesized to relate to possible deficits in attention or memory.

To counteract the confound of a rats' natural tendency to alternate, Johnson et al. (2002) studied rats with SAP infusions of the MS on a DMTP T-maze task. Rats received infusions of a high or low dose of SAP into the MS prior to behavioral testing. Upon recovery, rats were introduced to the DMTP task. The task required rats to revisit the same arm of the T-maze that they had entered on the previous trial. Rats that satisfied this requirement were provided with reinforcement and the trial was recorded as a correct choice. Daily performance (% of correct choices) and the number of days to criterion, (defined as 15 correct choices out of 16 consecutive trials), were calculated for each group. Once criterion was met, rats were given a variable delay (45, 60, 90 s) between the initial arm choice and the succeeding matching arm choice in a test of extended spatial working memory.

The results revealed that the control group's, but not the SAP lesioned group's percentage of correct choices on the first day of DMTP testing was well below chance levels; this lower than chance level of performance on the initial day of DMTP testing in control rats further

supports the view that normal rats have an innate tendency to alternate. In contrast, SAP lesions of the MS allowed the lesioned groups to perform better than controls on this initial day of DMTP testing. These results indicate that cholinergic lesions of the MS disrupt the natural tendency of alternating behavior in the rat. Despite this slow performance of controls early on, the control group, as compared to SAP lesioned rats, maintained a higher percentage of correct choices across days and were able to reach the imposed criterion in fewer days. Additionally, there were no significant differences between groups on the delay phase of the task. In general, the results were viewed as reflecting an impaired acquisition of the DMTP task following SAP lesions of the MS. The MS lesioned group took longer to learn the task and improved slower than controls. The results additionally revealed a dose-related deficit on the DMTP. That is, the higher dose SAP group performed worse than the lower dose SAP group across testing. The investigators acknowledge that the greater impairment in the high dose may be attributable to the greater extent of cholinergic basal forebrain damage observed in the high dose SAP group. Furthermore, the comparable performance between groups on the delay phase

of the DMTP task was viewed as evidence that MS lesions fail to produce deficits in spatial working memory.

Attention Perspective

In addition to the literature addressing the potential involvement of the cholinergic MS/VDB in spatial learning and memory, several reports suggest that SAP lesions of the MS/VDB in rats result in impaired attention (Baxter, Holland, & Gallagher, 1997; Lehmann et al., 2003). For example, Baxter et al. (1997) explored potential changes in attentional processing of conditioned stimuli in rats with SAP lesions of the MS/VDB. Following surgery, rats were tested on either a latent inhibition task or a serial conditioning task to assess attentional processing of conditioned stimuli. The testing procedures for each task were similar to those utilized by Chiba et al. (1995) to assess attentional processing of conditioned stimuli following SAP lesions of the NBM as previously discussed. The results of the latent inhibition task indicated that, following conditioned stimulus preexposure, during the conditioning phase, the amount of conditioned responding (i.e., food cup responding) was higher in the MS/VDB lesioned group than the control group. However, during the conditioning phase when the conditioned stimulus was novel (i.e., not preexposed), the

MS/VDB lesioned and control groups had similar response curves (i.e., no difference in conditioned responding observed). Moreover, the response curves during the conditioning of novel and preexposed stimuli were equivalent in the MS/VDB lesion group. The finding that latent inhibition does not occur normally after SAP lesions of the MS/VDB was viewed as indicating that the ability to decrease attention to conditioned stimuli (i.e., to disattend) is affected in these animals.

In a separate group of rats tested by Baxter et al. (1997) on a serial conditioning task, the results revealed that the amount of conditioned responding (i.e., food cup responding) was higher in the MS/VDB lesioned group as compared to controls when the predictive value of the conditioned stimuli was held constant. There was no difference between groups when the predictive value of the conditioned stimuli was adjusted. The results that MS/VDB lesioned rats conditioned responding is higher than controls when the predictive value of the conditioned stimuli is held constant indicates that decreases in attention to the conditioned stimuli normally seen following repeated presentations of predictive stimuli was disrupted.

In summation, there was a lack of latent inhibition along with a failure to decrease attention to conditioned stimuli when the predictive value was held constant following SAP lesions of the MS/VDB in the Baxter et al. (1997) report. These results were interpreted as suggesting that decreased attention to conditioned stimulus processing is critically influenced by the cholinergic projections of the MS/VDB. The results of Baxter et al. (1997) contrast those of Dougherty, Salat, and Walsh (1996), who found no deficits in latent inhibition following SAP lesions of the MS. However, both studies utilized different tasks to test latent inhibition; Dougherty et al. (1996) used a taste aversion paradigm, whereas Baxter et al. (1997) used visual stimuli and food cup behavior as the conditioned stimuli and conditioned response, respectively.

To broaden the understanding of the attentional processes affected by SAP lesions of the MS/VDB, Lehmann et al. (2003) investigated the effects of intraparenchymal SAP lesions of the MS/VDB in rats on a visuospatial attention taxing 5CSRT task. The testing procedures for the task were similar to those utilized by McGaughy et al. (2002) to assess visuospatial attention following SAP lesions of the NBM as previously discussed. The results

of the Lehmann et al. (2003) study revealed impaired postoperative performance on the 5CSRT task in rats with SAP lesions of the MS/VDB. Specifically, MS/VDB SAP lesioned rats, as compared to controls, demonstrated a postoperative decrease in response accuracy (i.e., the ability to detect signals) when the duration of stimulus presentations were reduced. Additionally, the MS/VDB lesioned group postoperatively emitted more premature responses (i.e., response disinhibition) than controls when stimulus presentations were made unpredictable by varying event rate (i.e., randomizing the duration of the inter-trial interval). The finding that SAP lesions of the MS/VDB results in deficit performance on the 5CSRT task when the attentional load of the task is increased provides support to the view that the cholinergic MS/VDB plays a critical role in the detection of significant stimuli in situations requiring sustained attention.

Combined Nucleus Basalis Magnocellularis and Medial Septum/Vertical Diagonal Band

Combined basal forebrain (i.e., NBM + MS/VDB) lesions using the selective immunotoxin SAP can produce or amplify impairments in behavior where separate NBM or MS/VDB lesions may produce modest deficits or fail to reveal any

behavioral impairments. The experiments described below further demonstrate this point.

Pizzo, Thal, and Winkler (2002) investigated the involvement of the entire cholinergic basal forebrain, including the cortically-projecting NBM and the hipocampally-projecting MS, on various behavioral tasks using rats that received combined intraparenchymal SAP injections into the NBM and MS. Following recovery from the combined intra-NBM/MS SAP lesion, rats were tested on a battery of behavioral paradigms, which included MWM spatial task, inhibitory avoidance, non-matching to position (NMTP) and matching to position (MTP) in a T-maze, and an elevated plus-maze (EPM) task. Spatial acquisition (i.e., finding a hidden platform located in the same position) of the water maze was unimpaired following intra-NBM/MS SAP lesions. On the other hand, as compared to control performance, spatial retention in the water maze task was impaired in the intra-NBM/MS group. Intra-NBM/MS rats did not remember the location of the hidden platform as well as controls when retested on the same task ten days after the last acquisition trial. Additionally, intra-NBM/MS rats demonstrated deficits in the water maze task when the platform was removed (spatial probe) 1 week after retention testing, indicating impaired

learning of the spatial location of the platform, despite apparently normal acquisition performance. The percentage of the total distance swam in the correct spatial location was significantly lower in the intra-NBM/MS group than controls on the probe trial when the platform was removed. Similarly, NBM/MS lesioned rats were impaired on the final spatial working memory portion of the water maze task where the platform was moved to a new location each day. Overall, the NBM/MS lesioned group swam a greater distance between daily trials than did controls when the platform was relocated to a new daily location, indicating a spatial working memory deficit.

On the inhibitory avoidance test, the NBM/MS lesioned rats remembered to avoid the negative stimulus and were therefore unimpaired on the task. Conversely, the NBM/MS lesion group was severely impaired on both the NMTP and MTP T-maze tasks. On both tasks, the acquisition curves of NBM/MS lesioned rats, compared to controls, were impaired as revealed by the greater amount of time required to reach criterion. In addition, the NBM/MS lesion group required significantly more trials to reach criterion on both tasks. When a delay (4, 30, 60, 90 s) was added to each task, only mild impairments were revealed in the NBM/MS lesion group. A significant

decrease in the mean percentage of correct responses was demonstrated only at the 30 s delay during NMTP testing and only at the 60 s delay during MTP testing, suggesting that spatial working memory was not dramatically impaired in either NMTP or MTP tasks.

Lastly, NBM/MS lesioned rats spent a greater amount of time in the open arms of an EPM as compared to controls. This EPM result may be interpreted as reflecting either a decrease in anxiety or as an impairment in spatial memory as reported by others (Lamprea et al., 2000). It is worth noting that individual SAP lesions of the NBM or MS did not impair performance on any of the above behavioral measures. Therefore, it was viewed that different types of behavioral impairments may be only revealed after significant reductions in cholinergic activity throughout the entire basal forebrain system is achieved.

Dornan et al. (1997) also studied the performance of rats with combined intra-NBM/MS SAP lesions on two spatial tasks, the MWM and the RAM. After recovery from surgeries, rats were first tested on the water maze followed by radial maze testing. Contrary to the findings of Pizzo et al. (2002), combined NBM/MS lesions failed to reveal any deficits in water maze performance. Combined

NBM/MS lesioned rats, as compared to controls, demonstrated similar escape latencies and path lengths on both noncued (spatial learning) and cued trials (non-spatial learning) as well as comparable probe trial (spatial retention) performance. In contrast, combined NBM/MS lesions impaired performance on the RAM task. In a partially baited eight arm radial maze combined NBM/MS lesioned rats made considerably more spatial working (recent) memory errors (i.e., reentries into previously baited arms) across testing than did controls, but there was no difference in spatial reference memory errors (i.e. entry into arms that were never baited) between groups. Interestingly, single SAP lesions of the NBM or MS produced similar, but milder deficits on the RAM task and a similar lack of impairment on the water maze. Taken together, the results suggested a disruption in spatial learning (only in the RAM) following cholinergic basal forebrain lesions, but this disruption was moderate compared to past research utilizing less selective lesion techniques.

CHAPTER THREE

NEUROBIOLOGICAL BASES OF DIFFERENTIAL REINFORCEMENT OF LOW RATE RESPONDING

In the experiments described in Chapter 4, the potential role of the rat basal forebrain cholinergic system (BFCS) in the acquisition and performance of either a standard (uncued) version or a cued version of a (free-operant) behavioral paradigm known as differential reinforcement of low rate responding (DRL) is explored. The DRL paradigm provides a measure to evaluate both timing and response inhibition. In the standard DRL task, each trial consists of a designated interval of time which must elapse without the animal responding, with reinforcement following the first response after the interval. If the animal responds before the interval is complete, the animal is not reinforced and the interval is reset (for a review see Kramer & Rilling, 1970). For example, in a DRL 20 second (DRL 20 s) task animals are only reinforced if a response is withheld for 20 s or longer from a previous response. If any response occurs before the 20 s time interval has elapsed, the interval is reset and responses must again be withheld for 20 s (or longer) in order to obtain reinforcement for the next

response. DRL responding is commonly analyzed through use of an inter-response time (IRT) frequency distribution. An IRT is simply the time between two responses. IRTs are usually separated into time intervals of 2 seconds, but it is up to the researcher to set the desired time interval. In the case of an IRT distribution with 2 s time intervals, for example, all responses with IRTs from 0 to 2 s are grouped into the first category (or IRT bin), all IRTs from 2 to 4 s are grouped into the second IRT bin, and so forth. When DRL responding is recorded this way, a bimodal curve usually forms with the first peak mode occurring at responses in the first IRT bin and a second peak mode occurring at the first IRT bin that is reinforced.

Previous research has implicated the frontal cortex, hippocampus, and septum in supporting differential DRL behavior in rats. Many experimental findings have demonstrated that lesions to these areas disrupt DRL performance. In regard to frontal cortical lesions, the findings have yielded conflicting results concerning the influence on DRL performance. Some studies have reported impaired DRL performance following frontal cortex lesions in rats (Kolb, Nonneman, & Singh, 1974; Nalwa & Rao, 1985, 2001; Neill, 1976; Numan, Seifert, & Lubar, 1975;

Rosenkilde & Divac, 1975), while others have reported that frontal cortical lesions produce no change or only slight deficits on DRL performance (Burkett & Bunnell, 1966; Finger et al., 1987; Kolb et al., 1974; Neill, 1976; Nonneman, Voigt, & Kolb, 1974; Schmaltz & Isaacson, 1968). Such inconsistencies in these studies may be attributed to the lesion techniques used (e.g., electrolytic or aspiration), which can vary significantly in size and/or location.

Of the studies that revealed DRL deficits, Numan et al. (1975) demonstrated that damage to the medial frontal cortex in rats impaired DRL performance. By the end of the DRL 20 s acquisition task, medial frontal lesioned rats emitted more responses and received fewer reinforcements causing medial frontal lesioned rats to perform less efficiently than controls. However, when a visual cue that signaled reinforcement was added to the task, the DRL deficit that was present during acquisition dissipated. The results were interpreted to suggest that medial frontal lesioned rats fail to regulate responding on the DRL in the absence of exteroceptive cues.

Additionally, Rosenkilde and Divac (1975) found that performance on a preoperatively trained DRL 10 s task deteriorated following anteromedial frontal cortex

lesions. By the end of postoperative DRL testing, anteromedial frontal lesioned rats emitted more responses, obtained fewer reinforcements, had a more evenly dispersed IRT distribution with peak response intervals that were more likely to be shifted to shorter IRTs. Similarly, Kolb et al. (1974) found that rats with orbital frontal cortex damage were more apt to make a second response within the first 2 seconds following a first response (i.e., 0-2 s IRT bin) on a DRL 20 s task. Orbital frontal lesioned rats were also more resistant to bar press extinction following DRL training. The explanation of these results was that perseveration on DRL and bar press extinction results from orbital frontal lesions.

Nalwa and Rao (1985, 2001) similarly found that DRL 10 s performance was disrupted by medial frontal cortical ablation in rats. The lesions resulted in a significant increase of nonreinforced premature responses (0-2 s IRT bin), although overall reinforcement rate remained relatively constant. Given that the number of reinforcements obtained was unaffected following medial frontal lesions, it was suggested that the results observed do not reflect a generalized timing deficit in these animals. Instead the increased response rate at shorter IRTs following medial frontal damage was viewed as

impair performance on a DRL 20 s schedule of reinforcement task in rats. Hippocampus lesioned rats emitted a greater number of responses, which consequently caused these animals to perform less efficiently than controls. The deficits observed on the DRL task in hippocampus lesioned rats were viewed as an inability to withhold responding during delay intervals (i.e., perseveration resulting from response disinhibition). The tendency to perseverate might similarly explain the greater resistance to extinction observed when this brain area is damaged.

Others have compared the performance of rats following excitotoxic lesions of designated areas in the hippocampal formation on the DRL paradigm (Sinden et al., 1986). Lesions of the complete hippocampus (CHC), which included areas CA1-CA4 and the dentate gyrus; lesions of the CA3 hippocampal region, which is the source of the major rostrally directed projections from the hippocampus; or lesions of the subiculum (SUB), which contains target cells for the major caudally directed projections from the hippocampus, were made with bilateral injections of ibotenic acid. Following recovery, rats were tested on a DRL 12 s task, shifted to a DRL 18 s task, and then placed in a DRL 18 s drug experiment. On the drug portion of the experiment, lesioned rats were given injections of saline

or the centrally acting anticholinergic agent scopolamine hydrobromide (Scop-HBr) and then placed on the DRL 18 s task.

The results of DRL 12 s testing indicated that efficiency was slightly impaired in all lesion groups. In addition, the response probability distribution (a plot of the probability of a response occurring at any given time interval) revealed that all groups formed a timing curve, indicating that the ability to time was unimpaired in these animals. The response probability distribution has been argued to be an unbiased estimate of temporal discrimination (i.e., timing) on the standard DRL task. As the delay between responses increases on the response probability distribution, the probability of responding gradually increases up to and slightly past the reinforcement interval in animals demonstrating temporal discrimination.

During the DRL 18 s task, efficiency was revealed to be most impaired in the CHC group. Moreover, the response probability distribution once again demonstrated a timing curve in all groups on the DRL 18 s task. The one difference was that the timing curve of the CHC group was shifted to the left (i.e., rats tended to respond at intervals of less than 18 s) relative to the other groups.

These findings were viewed as suggesting that any one lesion of the hippocampal formation only modestly impaired acquisition of the DRL 12 s schedule. Conversely, similar experiments using aspiration lesions of the CHC showed clear impairment on the DRL 12 s task (Rawlins et al., 1983), whereas in the Sinden et al. (1986) study only a slight impairment on the DRL 12 s schedule was found in the CHC group. Even though equivalent areas of the CHC are destroyed with excitotoxic lesions compared to aspiration or electrolytic lesions, the behavioral deficits are not as dramatic following excitotoxic lesions, maybe due to the sparing of fibers or terminals in the region associated with this method (Kohler & Schwarcz, 1983).

During drug testing on the DRL 18 s task in lesioned rats tested in the Sinden et al. (1986) study, the CHC group performed least efficiently, and the CA3 and SUB groups performed at an intermediate efficiency level as compared to controls. Scop-HBr treatment lowered efficiency in all groups. The response probability distribution showed that there was a timing curve on saline days in all groups; however, scopolamine treatment abolished the timing curve by increasing the probability

of early responses and decreasing the probability of late responses.

Because scopolamine reduced efficiency and changed the timing curve in the same manner in all lesioned groups, it appears that the effects of the drug simply sum with the effects of the lesion. The scopolamine effect, a flattening of the timing curve, was clearly different from the lesion effect, a shifting of the timing curve to the left in the case of the CHC group. The cholinergic blockade produced by scopolamine, therefore, may exert its influence extrahippocampally, quite possibly in the neocortex. Sinden and colleagues (1986) conclude that even though DRL schedules are sensitive to septohippocampal damage, it is the extent of the damage to the septohippocampal system, rather than the location of the lesions per se, that determined the degree to which DRL performance was impaired.

In a related study, performance of rats on a DRL 20 s task was compared following electrolytic lesions of designated areas in the hippocampus and its inter-related structures (Johnson et al., 1977). Electrolytic lesions of the entorhinal cortex (EC), posterior hippocampus (PH), anterodorsal hippocampus (AH), total fornix (TF), medial fornix (MF), lateral fornix (LF), postcommissural fornix

(PF), or septum (S) were created by passing radio-frequency current through an electrode into the targeted area.

The results of the study indicated that the TF group's postoperative efficiency was most impaired and recovered the least of any group. In the AH, MF, and S groups, initial postoperative performance was impaired but recovered over testing although never to the same level of preoperative performance. The postoperative response probability distribution revealed a similar deficit in temporal discrimination. The impairment in the TF group was most apparent, followed by the MF and LF groups, and the S and AH groups. With the exception of the TF group, the standard response probability distribution of the remaining groups exhibited some temporal discrimination despite the DRL efficiency deficit.

The findings were viewed as reflecting a functional difference between the anterior and posterior areas of the hippocampus as it relates to DRL performance. PH or EC lesions did not produce a consistent postoperative DRL deficit. Conversely, AH lesions produced a DRL deficit throughout postoperative testing. Likewise, the anterior fiber connections to the hippocampal system are important for maintenance of DRL performance as revealed by the

finding that TF lesions resulted in a gross postoperative DRL deficit. MF lesions resulted in a DRL deficit throughout postoperative performance, while the results of LF lesions were ambiguous. PF lesions, in contrast to other fornix lesions, produced no DRL impairments. Lastly, septal lesions produced a postoperative DRL deficit.

Ellen et al. (1964) also compared the involvement of the septum and hippocampus on DRL performance in rats. Lesions to the septum, hippocampus, dorsal isocortex overlying hippocampus, or corpus callosum overlying septum were created by electrolytic lesion. The results of the study established that DRL 20 s performance was significantly impaired in septal lesioned but not hippocampal lesioned rats. Over the course of DRL 20 s testing, septal lesioned rats emitted more responses, and obtained fewer reinforcements than controls. In addition, at the end of DRL 20 s testing, the distribution of IRTs revealed that septal lesioned rats emitted a greater number of responses within the first 5 seconds following a first response (i.e., 0-5 s IRT bin) compared to the other groups.

Furthermore, at the conclusion of DRL testing, the response probability distribution demonstrated that all

groups except the septal lesioned group formed a typical timing curve. In regard to septal lesioned rats, the response probability distribution revealed that timing behavior was impaired during short delay intervals (i.e., 0-10 s) but not long delay intervals (i.e., 11-25 s). Specifically, septal lesioned rats had a high and relatively equal probability of making a reponse 0-10 s after a preceding response, suggesting that within this time period responses were random. However, when delays between responses were longer (i.e., 11-25 s), the probability of septal lesioned rats responding also gradually increased, indicating that at longer delays temporal discrimination (i.e., timing) was unimpaired.

These findings were viewed as suggesting that the DRL impairment in rats with septal lesions may be related to a loss of response inhibition of goal-directed behavior. Particularly, septal lesioned rats exhibited random responding at short delay intervals but not long delay intervals, which suggests that septal lesioned rats were unable to inhibit responding rather than suffering from a generalized timing deficit. The authors additionally suggested that because the primary septal efferents project to the mamillary body, hippocampus, anterior thalamic nuclei, and gyrus cinguli, and given the lack of

DRL impairment following hippocampal, callosum-cingulate, and isocortical damage, then the DRL impairment may be mediated via the septal efferents to the mamillary body and anterior thalamic nuclei.

Of the three areas (frontal cortex, hippocampus, and septum) previously implicated in the control of DRL behavior, it appears that damage to the septal area reveals deficits on the DRL paradigm more consistently than damage to the other two structures. In fact, of the two preceding studies which examined and compared DRL performance of both septal and hippocampal lesioned rats in the same experiment, septal lesioned rats were found to be impaired in both studies (Ellen et al., 1964; Johnson et al., 1977), while hippocampus lesioned rats were found to be impaired in only one of the studies (Johnson et al., 1977).

A number of studies have additionally corroborated these findings in that rats with lesions to the septum are typically sensitive to DRL parameters (Brookes, Rawlins, Gray, & Feldon, 1983; Burkett & Bunnell, 1966; Kelsey & Grossman, 1971). For example, Brookes et al. (1983) found that DRL 20 s performance was impaired following electrolytic lesions of the medial or lateral septal area. At the end of DRL 20 s testing, both septal lesioned

groups continued to emit more responses and perform less efficiently than controls. Despite these DRL impairments, both septal lesioned groups were able to develop a clear timing curve as reveled by their response probability distributions. The lack of timing deficit per se, along with the increased responding observed on the DRL task, led the researchers to interpret the findings as reflecting a deficit in response inhibition.

Burkett and Bunnell (1966) found that the retention of a preoperatively trained DRL 20 s task to be impaired following electrolytic septal lesions in rats. Following septal lesions, rats increased response rate and concomitantly decreased reinforcements obtained. The researchers similarly interpreted the DRL impairment as impairment in the ability to inhibit goal directed responses (i.e., response disinhibition), most likely as a result of interference in the septohippocampal system.

In addition, Kelsey and Grossman (1971) indicated that electrolytic lesions of the septal area in rats disrupt acquisition of a DRL 30 s task. Although the septal lesion group showed an increased response rate only over the first few days of testing, they obtained fewer reinforcements throughout the course of DRL testing. Moreover, when an external auditory cue that signaled the

availability of reinforcement was added to the task, the septal lesion induced impairment was ameliorated. In the presence of the cue, septal lesioned rats were able to obtain as many reinforcements as controls. The difference between cued and uncued DRL performance in septal lesioned rats was viewed as an inability to inhibit responding in the absence of an exteroceptive cue in the uncued DRL task.

Kelsey and Grossman (1971) are not the only researchers to observe an attenuation of uncued DRL deficits in lesioned rats following the addition of an external cue indicating reinforcement availability. Similar DRL findings have been demonstrated in rats with frontal cortical lesions (Numan et al., 1975), hippocampal lesions (Braggio & Ellen, 1976; Pellegrino & Clapp, 1971; Rickert, Bennett, Anderson, Corbett, & Smith, 1973), and septal lesions (Ellen & Butter, 1969; Braggio & Ellen, 1976). For example, in corroboration with other studies, Pellegrino and Clapp (1971) found rats with aspiration lesions of the hippocampus to be impaired on the acquisition of a standard uncued DRL 20 s task. Hippocampal lesioned rats emitted a higher rate of responding and a lower percentage of correctly timed responses compared to controls. Although performance was

severely impaired on the uncued DRL task, no such deficits were observed in hippocampus lesioned rats when a visual cue indicating reinforcement availability was present at the start of DRL testing.

The findings were viewed as indicating that rats with hippocampal lesions are able to withhold or inhibit responding when an external cue is present but not when it is absent. An earlier report using rats with septal lesions suggested that uncued DRL deficits might be the consequence of a failure to utilize internal cues such as response-produced, proprioceptive stimuli as cues for lever pressing (Ellen & Butter, 1969). An alternative interpretation of the uncued DRL deficits in hippocampal lesioned rats focused on task difficulty. Comparisons of control rats' performance on the cued and uncued DRL task revealed that the uncued condition is more difficult to learn than the cued condition. Consistent with this interpretation, deficits in the hippocampal lesioned rats on the uncued condition may therefore result from a general learning deficit, which is a function of task difficulty rather than a failure to inhibit responding.

Rickert et al. (1973) reported similar findings when rats with aspiration lesions of the hippocampus were tested on uncued and cued DRL schedules. In that

experiment, one group of lesioned rats was tested on one of three DRL schedules (10, 20, or 30s) in the absence of a visual cue, while the other group was provided with a cue signaling reinforcement availability. The results of the uncued condition indicated that the hippocampal lesioned group, relative to controls, was impaired on the DRL regardless of schedule (10, 20, or 30 s), as revealed by the lower proportion of correct responses emitted across sessions. In the cued condition, hippocampal lesioned rats are initially impaired but are ultimately able to achieve the same levels of performance as controls irrespective of DRL schedule.

The results of the study were viewed as a failure to support the behavioral inhibition hypothesis of hippocampus function. Although results of the uncued condition are consistent with previous reports showing a response disinhibition in hippocampal lesioned rats under uncued DRL conditions (Clark & Isaacson, 1965), there was no change in magnitude of deficit (i.e. increase in response rate) as the DRL schedule increased and became more stringent. There is a possibility that the hippocampus damaged rats are so severely impaired on the intermediate schedule that an increase in the IRT requirement may not reveal any additional deficits.

Moreover, the cued condition provides further convincing evidence against the behavioral inhibition hypothesis of the hippocampus. Although initial performance was disrupted in hippocampectomized rats on the cued DRL schedules, final performance was comparable between hippocampus lesioned and control groups.

Pellegrino and Clapp (1971) have suggested that the differences in performance of hippocampal lesioned rats on cued and uncued DRL tasks reflects an inability to use response-produced stimuli for succeeding responses or a general learning deficit that covaries with task difficulty. The results of Rickert et al. (1973), however, are contrary to the view that hippocampal lesioned rats experience a general learning deficit as indicated by comparable performance on less (DRL 10) and more (DRL 20) demanding schedules. Therefore, the findings instead suggest an inability of hippocampal lesioned rats to use response-produced stimuli for succeeding responses.

Braggio and Ellen (1976) examined both uncued and cued DRL performance after electrolytic lesions to the septum or other anatomically related structures in rats. The results of the study revealed an increase in responding in rats with lesions of the septum and

hippocampus on the initial acquisition of the standard uncued DRL task. However, the permanence of this overresponding symptom differed according to lesion site and DRL parameters. For example, septal lesioned rats overresponded on the standard DRL even when given a prior history of training on the cued DRL suggesting that overresponding is a primary dysfunction of the septal lesion. However, it should be noted that septal lesioned rats were able to reduce response rate when an external cue signaling reinforcement was present.

Conversely, hippocampal lesioned rats were able to improve subsequent performance, after the removal of the external cue, emitting fewer total responses and increasing reinforcements obtained as compared to hippocampal lesioned rats that remained on the standard uncued DRL. Thus, the overresponding symptom in hippocampal lesioned rats may not be a primary dysfunction of the lesion. Rather, the symptom is secondary to an inability to inhibit responding to an established response pattern, otherwise known as perseveration. Other studies have shown that an overresponding symptom on DRL occurs in hippocampal lesioned rats only with previous training on a continuous reinforcement schedule (Ellen, Aitken, & Walker, 1973; Schmaltz & Isaacson, 1966), suggesting a

perseveration impairment in animals with hippocampus damage (Braggio & Ellen, 1976).

The overall findings were viewed as reflecting that overresponding on the DRL in rats with lesions to the septum and hippocampus can be attenuated with the presence of an external cue. The cue may therefore be acting as a discriminative stimulus for lever pressing in which case the cued DRL would be reduced to a simple light/dark discrimination. Another interpretation of the cued DRL is that in this experiment it may function as a time-out from standard DRL testing. This interpretation is supported by the data indicating that normal rats, which received exposure to and then removal of the cued DRL never achieved the same level of performance as normals on the standard DRL alone.

Pharmacological studies have shown that anticholinergic drugs influence DRL performance in rats as well (Kelsey & Grossman, 1975; Meyer, Severson, & Thompson, 1976; Soffie & Lejeune, 1992). Meyer et al. (1976) found that the centrally acting anticholinergic drug scopolamine interferes with DRL performance in rats. The effect of scopolamine was shown by the significantly lower number of reinforcements obtained, which was a function of the increase in the number of nonreinforced

responses. Soffie and Lejeune (1992) similarly reported that scopolamine (0.5 mg/kg) increased the perseveration of nonreinforced responses, in turn reducing the percentage of reinforcements obtained. Additionally, Kelsey and Grossman (1975) have reported that systemic injections of scopolamine impaired performance on a DRL 30 second task, by increasing responses, and consequently, reducing the rate of reinforcement.

CHAPTER FOUR

THESIS EXPERIMENTS

In these experiments, the role of the rat basal forebrain cholinergic system (BFCS) in the acquisition and performance of either a standard (uncued) version or a cued version of the differential reinforcement of low rate responding (DRL) task with a limited-hold (LH) contingency is explored using selective lesions of the BFCS made by infusing the cholinergic immunotoxin 192 IgG-saporin (SAP). The BFCS is comprised of the cholinergic nucleus basalis magnocellularis (NBM), which projects to the neocortex, and the medial septum/vertical diagonal band of Broca (MS/VDB), which projects to the hippocampus. The standard DRL paradigm assesses both timing and response inhibition. In the standard DRL task, each trial consists of a designated interval of time which must elapse without the animal pressing a lever, with reinforcement following the first response after the required interval. If the animal responds before the interval is complete, it is not reinforced and the interval is reset (for a review of the DRL procedure see Kramer & Rilling, 1970). In the case of a DRL 20 s task animals are only reinforced if responding is withheld for 20 s or longer since the previous response

was emitted. If any response occurs during the 20 s time interval, the timer is reset and responses must again be withheld for 20 s (or longer) in order to obtain reinforcement for the next response. The addition of a LH contingency to the DRL task forces animals to time their responses with greater precision than in the standard DRL task where no upper limit is set for responding. In the DRL LH task, animals must not only learn to refrain from responding for a specific interval of time, as in the standard DRL, but they must also learn not to wait longer than the designated time interval before responding in order to receive reinforcement. In the case of a DRL 20 s LH 10 s task, animals are only reinforced for responding during the 10 s window occurring 20-30 s from the most recent response. If a response occurs outside this 20-30 s reinforcement window, the interval is reset and responses must again be withheld for 20-30 s in order to obtain reinforcement.

Previous research suggests that the brain systems implicated in supporting DRL behavior include the medial prefrontal cortex (Nalwa & Rao, 1985, 2001), medial septum (Ellen et al., 1964), and hippocampus (Braggio & Ellen, 1976; Clark & Isaacson, 1965). Lesions of each of these structures typically impair DRL performance because of

perseveration (i.e., an increase in responding at short inter-response intervals). Perseveration may result from lesion-induced disruptions in the ability to sustain attention to temporal processing or response inhibition required in the uncued DRL task. Given that both the medial prefrontal cortex and the hippocampus receive projections from the BFCS, and that the medial septum is itself part of the BFCS, it is possible that cholinergic modulation of these brain regions contributes to normal DRL performance. This argument is supported by the finding that systemic administration of the centrally acting anticholinergic drug scopolamine interferes with uncued DRL performance (Kelsey & Grossman, 1975; Meyer, Severson, & Thompson, 1976; Soffie & Lejeune, 1992); scopolamine causes an increase in the number of nonreinforced responses and a resulting decrease in response efficiency as compared to controls. Because damage to the hippocampus, medial septum, or medial prefrontal cortex, as well as scopolamine treatment each impair uncued DRL performance, it is reasonable to infer that BFCS lesions, which similarly compromise cholinergic function, will lead to impairments in the DRL task.

In the current experiments, it was hypothesized that SAP lesions of the BFCS, including the NBM and MS/VDB,

would disrupt DRL acquisition and performance in the uncued DRL task by causing perseveration, where this deficit may reflect an impairment in the ability to sustain attention. Specifically, it was predicted that rats in the BFCS lesion group, compared to controls, tested in the uncued DRL paradigm would make a greater number of total lever presses, and a greater number of nonreinforced responses, especially at short inter-response intervals. Additionally, the BFCS lesion group was expected to show a correspondingly lower response efficiency than controls. Collectively, these predicted behavioral changes would reflect a perseveration impairment, perhaps caused by an underlying disruption in sustained attention. In contrast, no impairments in timing was predicted as suggested by previous research showing intact timing in rats with lesions of the medial prefrontal cortex (Nalwa & Rao, 1985, 2001), medial septum (Brookes et al., 1983), or the hippocampus (Ellen et al., 1964).

Given that the uncued DRL task is assumed to depend on both the ability to time responses and on the ability to inhibit responding during the inter-trial interval, an impairment in DRL performance could be indicative of either perseveration, impaired timing ability, or both.

However, previous research using the uncued DRL task suggests that damage to the hippocampus does not impair timing ability per se (Ellen et al., 1964). Instead, perseveration appears to underlie the DRL impairments seen in hippocampus damaged rats (Braggio & Ellen, 1976; Clark & Isaacson, 1965). Nevertheless, it remains possible that the extensive cholinergic depletion of both the hippocampus and cortex resulting from SAP lesions of the BFCS might impair timing in the current experiments. In order to further distinguish between possible impairments in timing or response inhibition, additional groups of BFCS lesion and control rats were tested on a cued version of the DRL task. This task requires response inhibition, but does not require response timing as does the uncued DRL task. Specifically, in the cued DRL task, animals must learn to respond to an external cue signaling the availability of reward during the 20-30 s reinforcement interval, thus precluding the explicit need for timing behavior. The cued DRL task, therefore, provides a measure of response inhibition that should be insensitive to potential timing requirements associated with the uncued DRL task.

Based on reports of rats with either hippocampus (Braggio & Ellen, 1976; Pellegrino & Clapp, 1971; Rickert

et al., 1973), septum (Ellen & Butter, 1969; Braggio & Ellen, 1976), or medial prefrontal cortex lesions (Numan et al., 1975) tested in a cued DRL task, where these animals performed as well as controls, it was hypothesized that rats in the BFCS lesion group would learn to withhold responding during nonreinforced intervals and would not show perseveration in the cued DRL task. Additionally, the BFCS lesion group was expected to learn to discriminate and respond to the food-reinforced cue as well as controls tested in the cued DRL task.

To summarize, it was hypothesized that performance in the BFCS lesion group would be impaired compared to controls in the uncued DRL task but would be spared in the cued DRL task. Such a differentiation in the effects of SAP lesions of the BFCS in the cued and uncued DRL tasks would demonstrate a role for the BFCS in inhibiting perseveration, but only in tasks requiring sustained attention to temporal or response inhibition requirements such as those found in the uncued task.

CHAPTER FIVE

METHODOLOGY

Materials and Methods

Animals

A total of 40 male Long-Evans rats (approximate weight 250 g upon arrival) were purchased from a commercial research animal vendor (Harlan Sprague-Dawley, Indianapolis, IN) and shipped to the animal facility in the Social & Behavioral Sciences Building on the campus of California State University, San Bernardino (CSUSB). Rats were individually housed under a 12 hr light/dark cycle (lights on at 1800). For a period of 3 weeks prior to shaping procedures, rats were allowed free access to food and water, and were handled for approximately 5 minutes daily. Following surgery rats were randomly assigned to each of the following groups: in the uncued differential reinforcement of low rate responding (DRL) task, a basal forebrain cholinergic system (BFCS) lesion group (n = 10), a sham-operated control group (n = 5), and an unoperated control group (n = 5); and in the cued DRL task, a BFCS lesion group (n = 10), a sham-operated control group (n = 5), and an unoperated control group (n = 5).

Apparatus

Testing was conducted in individual computer-controlled, sound-attenuating operant chambers (Coulbourn Instruments, Allentown, PA) equipped with a response lever located in the center of the front panel of the chamber, and a 7.5-W white cue light positioned directly above the response lever. Reinforcement consisted of 45 mg sucrose pellets (P. J. Noyes Co., Lancaster, NH) delivered into a magazine located at floor level to the left of the response lever. A 5-W white houselight located at the top of the rear wall of the chambers provided ambient illumination. The presentation of cue light, the delivery of reinforcement, and lever-press detection was controlled by means of a computer interface (WINLINC, Coulbourn Instruments, Allentown, PA).

Procedure

Shaping. Beginning 1 week before lever-press shaping, all rats were gradually reduced and then maintained at approximately 85% of their ad libitum feeding weights, with water freely available. Rats were shaped to lever press for sucrose pellets on a continuous reinforcement schedule for 5 consecutive days. Each daily shaping session was terminated either after the rat had pressed the lever 50 times and earned 50 reinforcers or

after 50 minutes had elapsed, whichever came first. Following each shaping or behavioral testing session, rats were fed a restricted amount of food (10-15g) immediately upon returning to their home cages to maintain them at 85% of their ad libitum feeding weights. After the fifth day of lever press shaping, rats were randomly assigned to each of the groups outlined above.

Surgery. Surgical procedures followed those used by Baxter et al. (1995) in their 192 IgG-saporin (SAP) lesions of the BFCS, including the nucleus basalis magnocellularis (NBM) and medial septum/vertical diagonal band of Broca (MS/VDB). Prior to surgery, rats received 65 mg/kg i.p. sodium pentobarbital anesthesia (Butler Co., Dublin, OH), sufficient to reach a surgical plane of anesthesia as determined by absence of response to tail-pinch, absent eye blink reflex. Surgery was performed under aseptic conditions. After shaving, cleaning (70% ethyl alcohol), and treating the scalp with a topical antibacterial solution (Betadine), the anesthetized animal was placed in a stereotaxic frame (David Kopf Instruments, Tajunga, CA) and the eyes were lubricated with opthalmic lubricant. A 1.5 cm incision was made in the scalp along the midline, the periosteum on the skull top was

deflected, and the surrounding skin and musculature was deflected laterally.

Using a stereotaxic drill (David Kopf Instruments, Tajunga, CA) with sterile bit, two craniotomies were made in the skull above the MS/VDB bilaterally at the following coordinates: +0.45 mm anterior to bregma and ±0.6 mm lateral to midline. A series of four additional craniotomies were made in the skull bilaterally above the NBM at the following coordinates: -0.75 mm posterior to bregma at ±2.3 and ±3.3 mm lateral to midline. Prior to intracerebral infusion of the immunotoxin SAP (or Dulbecco's sterile saline in sham-operated rats; Sigma Chemicals, St.Louis, MO), the dura beneath each craniotomy was opened using a sterile, fine-gauge needle tip to allow passage of infusion cannula.

Rats in the BFCS lesion group received a total of eight infusions of SAP (Chemicon, Temecula, CA) in Dulbecco's sterile saline solution at a concentration 0.4 µg/µl. A microinjection unit (David Kopf Instruments, Tajunga, CA), capable of delivering small volumes, was mounted to the stereotaxic frame. Using the microinjection unit, the SAP solution was infused via a 28-gauge, blunt-tip syringe (Hamilton, Reno, NV) at a rate of 0.1 µl/min, bilaterally into each MS/VDB and NBM site

referenced above. First, a volume of 0.3 µl SAP solution was infused into each MS/VDB site at a depth of -7.8 mm below the surface of the level skull, followed by the infusion of a volume of 0.2 µl SAP solution at a depth of -6.2 mm below the surface of the level skull. The cannula was left in place for an additional 6 min following each 0.3 µl infusion and 3 min following each 0.2 µl infusion to allow diffusion of the immunotoxin away from the cannula tip.

Next, a volume of 0.2 µl SAP solution was infused into each medial NBM site at a depth of -7.8 mm below the surface of the level skull, and into each lateral NBM site at a depth of -8.1 mm below the surface of the level skull. The cannula was left in place for an additional 3 min following each NBM infusion to allow diffusion of the immunotoxin away from the cannula tip.

Surgical procedures were identical for rats in the sham-operated control group, with the critical distinction that these animals received infusions of Dulbecco's sterile saline only.

All surgical instruments were sterilized prior to use by immersion in liquid sterilant and rinsed in sterile water. This sterilizing procedure was repeated between surgeries, as multiple surgeries were conducted on any

given surgical day. Animal preparation (weighing, shaving the scalp, etc.) was done in a clean room adjacent to the surgical suite.

Following surgery for rats in both groups (BFCS or sham-operated), the incision was cleaned and sutured and rats were returned to their home cages and allowed 7 days for recovery before behavioral testing. During recovery, the condition of the animals were closely monitored by the principal investigator and his laboratory personnel for at least 2 weeks post-op; assessment of general animal health and inspection of incision sites occurred daily and animals were weighed twice weekly as a means of assessing recovery.

Behavioral Testing. One week after surgery, rats were returned to shaping on the pre-operative continuous reinforcement schedule for 5 additional days before being shifted to either the uncued or the cued DRL task. In the uncued DRL task, rats were tested on a DRL 20 s interval with a 10 s limited-hold contingency (DRL 20 s LH 10 s) schedule of reinforcement. On this schedule, rats are only reinforced if they withhold responding for at least 20 s, but not for more than 30 s. Responses occurring outside this reinforcement time interval reset the interval and are not reinforced. There was 40 trials

(i.e., opportunities for reinforcement) per session, for 60 consecutive daily sessions. Each daily session concluded either after the rat received 40 reinforcers or after 50 minutes had elapsed, whichever came first. In the cued DRL task (DRL 20 s LH 10 s), testing parameters were identical to the uncued DRL task, except that rats in this task were given a 10 s visual cue (illumination of the light above the lever) signaling the interval in which reinforcement is available.

Euthanasia and Histology

Upon completion of behavioral testing, rats were administered a lethal dose of sodium pentobarbital (80 mg/kg, i.p.; Sigma, St. Louis, MO). Brains were extracted, sectioned, stained for acetylcholinesterase, and photographed to verify the extent of the lesions.

Statistical Analyses

Dependent variables for each session in both the uncued and cued DRL tasks included total number of responses (i.e., lever presses), and number of responses emitted within the first 2 s of a previous response (i.e., 0-2 s bin of inter-response times or IRTs), where these two variables provided a measure of perseveration. Total number of reinforced responses (bins 11-15), number of responses in the first reinforced time interval (i.e., bin

11), and number of responses in bins 12-15 were also analyzed to provide an assessment of rats' ability to time their behavior in the uncued DRL task or to discriminate the reinforcement cue in the cued DRL task. Finally, response efficiency (reinforced responses/total responses) was analyzed for each block of testing (5 days/block). The response efficiency ratio provides a measure of DRL performance that is insensitive to absolute differences in responding between animals, and instead measures relative DRL performance within each animal. Efficiency ratios near 1.0 reflect very good performance, with a minimal number of nonreinforced (i.e., premature) responses and a maximum number of reinforced (i.e., correctly timed) responses; efficiency ratios near 0.0 reflect poor performance with many nonreinforced and few reinforced responses.

The results from the uncued and cued paradigms were analyzed by separate mixed between-within analyses of variance (ANOVA) first comparing the unoperated and sham-operated control groups; because these two groups did not differ on five of the six separate dependent variable measures in the uncued DRL task, they were combined to form a single control group in the uncued DRL task. The unoperated and sham-operated control groups in the cued

DRL task did not differ on any measure and were similarly combined. Next, separate ANOVAs comparing the BFCS lesion group to the control group (between group factor), in each task, across blocks of test days (5 days/block; within group factor) was performed. Each dependent variable: total responses, number of responses in bin 1 (i.e., perseverative responses), total number of reinforced responses (bins 11-15), number of responses in bin 11 (i.e., correctly timed or discriminated responses), number of responses in bins 12-15 (i.e., relatively well timed or discriminated responses), and response efficiency was analyzed.

CHAPTER SIX

RESULTS

Unoperated Versus Sham-operated Control Group Comparisons

Statistical analyses in both experiments revealed that the unoperated and sham-operated control groups in both the cued and uncued DRL tasks were not different from one another (i.e., showed no significant interaction or between-group effects) on any of the dependent variable measures (total responses, bin 1 responses, total reinforcers, bin 11 responses, bins 12-15 combined responses, or efficiency scores), with only one exception. There was a significant interaction effect between the unoperated control group and the sham-operated control group on the number of bin 1 responses emitted in the uncued DRL experiment. The unoperated control group made more responses in bin 1 than the sham-operated control group on the first block of testing. However, from the second block on, the sham-operated control group made more responses in bin 1 compared to the unoperated control group. This difference appears to be the result of an unusually high bin 1 response rate in two animals in the sham-operated control group; it appears likely that with

more subjects this difference between sham-operated and unoperated groups would diminish.

Because the unoperated and sham-operated control groups in the uncued DRL task did not differ on five of six separate dependent variable measures, they were combined for all subsequent analyses. The unoperated and sham-operated control groups in the cued DRL task did not differ on any measure and were similarly combined for all subsequent analyses. The combined sham-operated and unoperated control groups are referred to as the control group from here on.

Uncued Differential Reinforcement of Low Rate Responding

Performance across blocks (5 days/block) of testing in the BFCS and control groups tested in the uncued DRL task is shown in Figures 1 and 2. A detailed analysis of the constituent dependent variables in this task follows.

Total Responses

The mean number of total responses emitted by the BFCS lesion group and the control group during uncued DRL 20 s LH 10 s training are shown in Figure 3. The number of total responses emitted during uncued DRL training decreased across blocks of testing in both groups. ANOVA

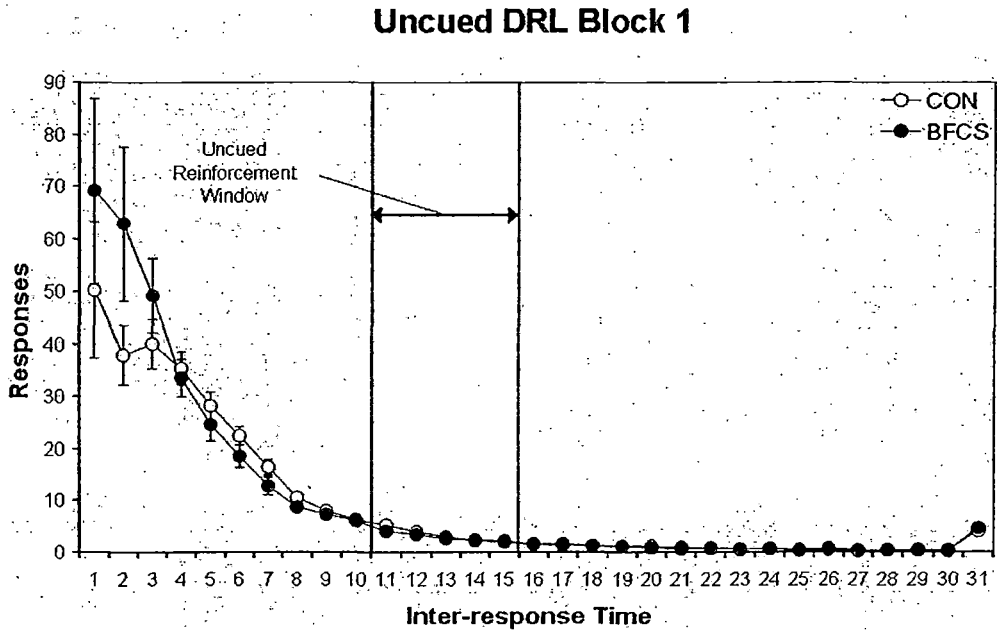


Figure 1. Mean (+/- SEM) Inter-response Time (IRT) Distributions (2 s per Bin) for the Basal Forebrain Cholinergic System (BFCS) and Control (CON) Groups in the Uncued Differential Reinforcement of Low Rate Responding (DRL) Task on Block 1 (Days 1-5). Responses made in bins 11-15 (i.e., 20-30 s IRTs) were reinforced. Note the greater number of bin 1 responses (i.e., perseveration) in the BFCS lesion group as compared to the control group.

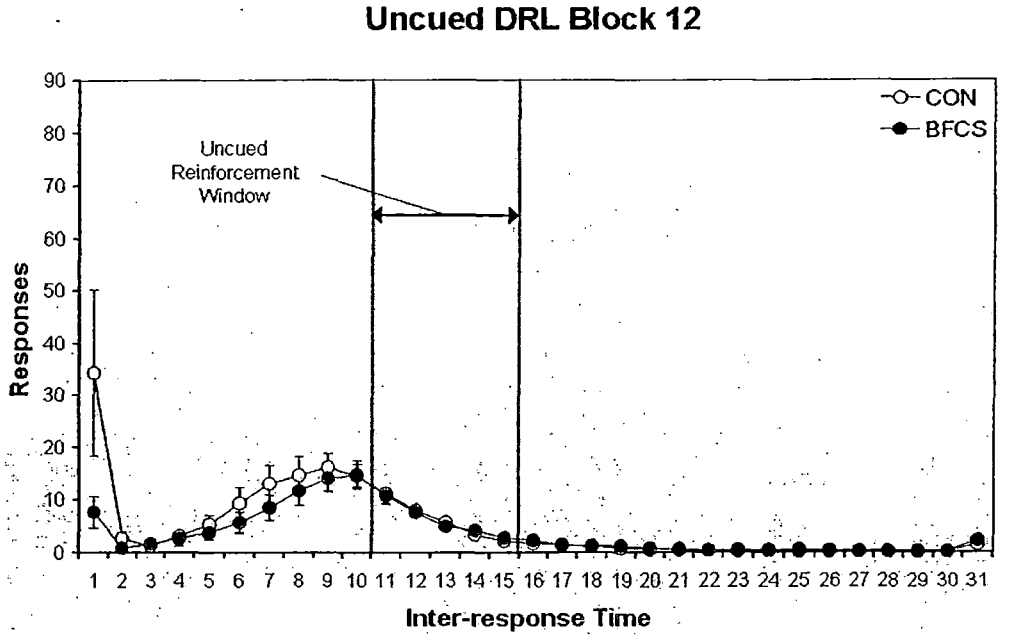


Figure 2. Mean (+/- SEM) Inter-response Time (IRT) Distributions (2 s per Bin) for the Basal Forebrain Cholinergic System (BFCS) and Control (CON) Groups in the Uncued Differential Reinforcement of Low Rate Responding (DRL) Task on Block 12 (Days 56-60). Responses made in bins 11-15 (i.e., 20-30 s IRTs) were reinforced. By the end of training, the BFCS lesion group no longer emits a greater number of bin 1 responses and instead the control group perseverates more than the BFCS lesion group. Note the emergence of timing behavior in both groups across the 12 blocks (5 days per block) of training; by the end of training, responding in both groups reaches a peak just before the reinforcement window begins.

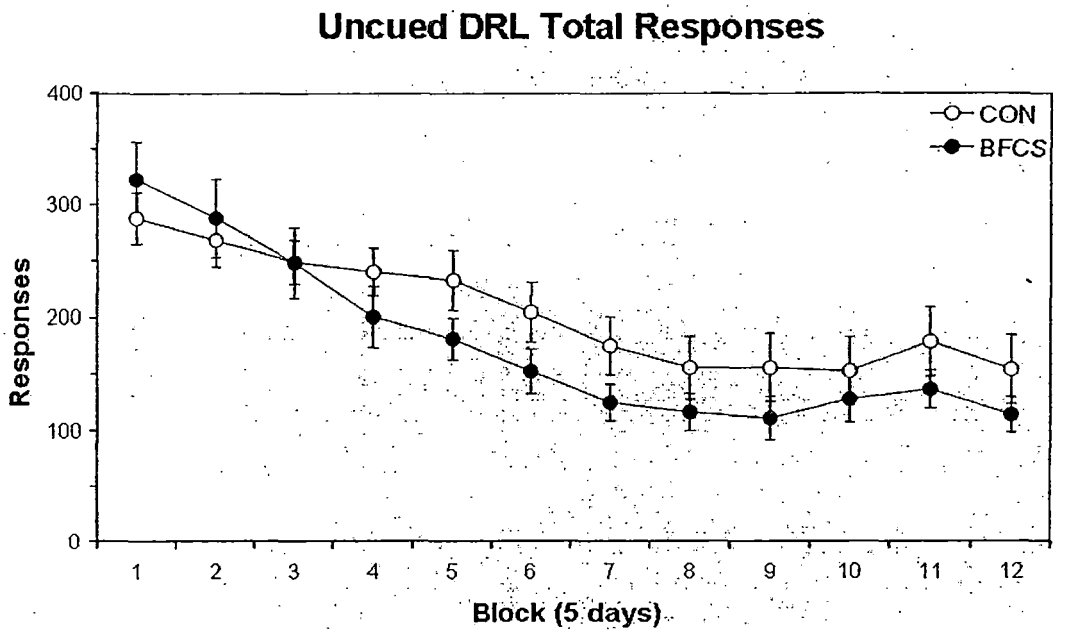


Figure 3. Mean (+/- SEM) Number of Total Responses across the Inter-response Time Distribution (i.e., Responses in Bins 1-31) for the Basal Forebrain Cholinergic System (BFCS) and Control (CON) Groups across Blocks (5 Days per Block) in the Uncued Differential Reinforcement of Low Rate Responding (DRL) Task. Perseverative responding in the BFCS and CON groups differed across testing blocks. Early in training, BFCS animals made more total responses than controls; later in training, however, BFCS animals made fewer total responses than controls (interaction effect, $p = .011$).

confirmed these observations; a main within-group effect for Block yielded an $F(11, 198) = 39.20$, $p < .001$. No interaction between linear trends associated with the two groups was observed, indicating that the rate of decrease in number of total response made across blocks did not differ between groups.

Although both groups showed a decrease in total responding across blocks of testing (as revealed by the significant main within-group effect) during uncued DRL testing, the number of total responses emitted did not change at the same rate across testing blocks in the BFCS lesion group and the control group. Early in training, the BFCS lesion group made more total responses than the control group; later in training, however, the BFCS lesion group made fewer total responses than the control group. ANOVA confirmed these observations; a Group by Block interaction yielded an $F(11, 198) = 2.317$, $p = .011$.

No significant between-group differences in number of total responses between the BFCS lesion group and the control group were observed on this measure.

Perseverative (Bin 1) Responses

The mean number of bin 1 responses emitted by the BFCS lesion group and the control group during uncued DRL 20 s LH 10 s training are shown in Figure 4. The number

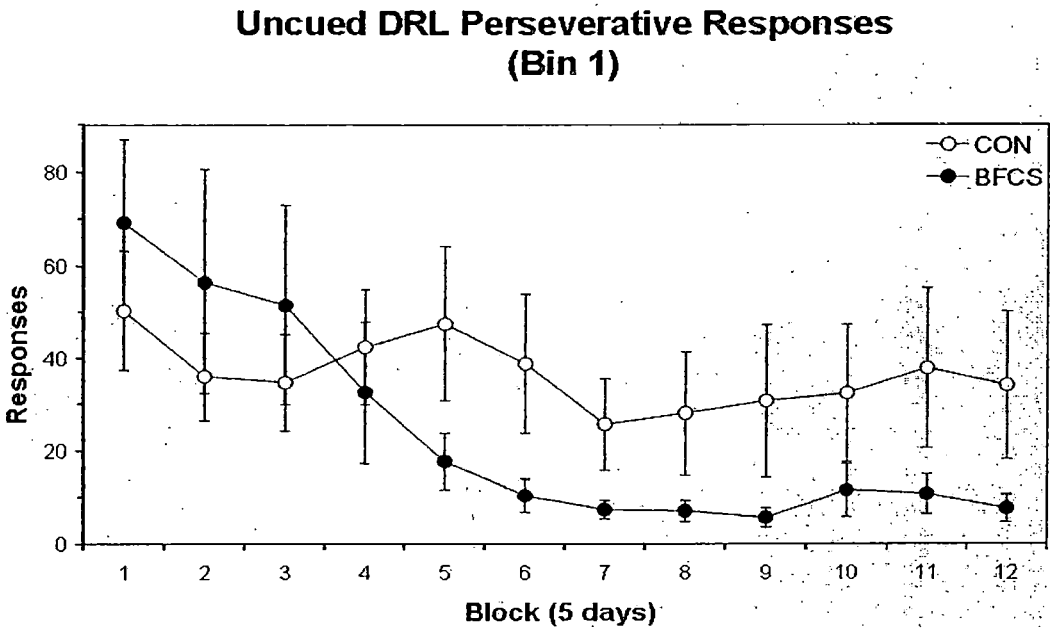


Figure 4. Mean (+/- SEM) Number of Short (0-2 s) Inter-response Time (IRT) Responses (i.e., Responses in Bin 1) for the Basal Forebrain Cholinergic System (BFCS) and Control (CON) Groups across Blocks (5 Days per Block) in the Uncued Differential Reinforcement of Low Rate Responding (DRL) Task. Perseverative responding in the BFCS and CON groups differed across testing blocks. Early in training, BFCS animals made more short IRT responses than controls; later in training, however, BFCS animals made fewer short IRT responses than controls (interaction effect, $p = 0.001$).

of responses emitted in bin 1 during uncued DRL training decreased across blocks of testing in the BFCS lesion group, but not the control group. ANOVA confirmed these observations; a main within-group effect for Block yielded an $F(11, 198) = 5.971$, $p < .001$, indicating a decrease in the number of responses emitted in bin 1 across testing blocks in one or both groups during uncued DRL testing. Linear trend analyses similarly indicated a within-group decrease in bin 1 responding across testing blocks ($F(1, 18) = 10.745$, $p = .004$). By itself, this linear trend effect for Block did not provide information as to which group was exhibiting a significant trend in uncued DRL bin 1 responding. Thus, within-group linear trends were analyzed separately for each group. These analyses revealed that the BFCS lesion group ($F(1, 9) = 9.923$, $p = 012$), but not the control group, showed significant linear trends in bin 1 responding across testing blocks. A linear trend interaction between the two groups' bin 1 responding across testing blocks ($F(1, 18) = 4.822$, $p = 041$) confirmed that the BFCS lesion group decreased bin 1 responding across blocks while the control group, which began with comparatively low bin 1 responding, did not show this effect.

A similar finding, where early in training the BFCS lesion group made more bin 1 responses and later in training the BFCS lesion group made fewer bin 1 responses than the control group is provided by a significant Group by Block interaction ($F(11, 198) = 3.066$, $p = .001$). Thus, the BFCS lesion group, but not the control group, showed a decrease in bin 1 responding across blocks of testing during uncued DRL testing.

No significant between-group differences in number of responses emitted in bin 1 between the BFCS lesion group and the control group were observed on this measure.

Total Reinforcers (Bins 11-15)

The mean number of total reinforcers obtained by the BFCS lesion group and the control group during uncued DRL 20 s LH 10 s training are shown in Figure 5. The number of total reinforcers obtained during uncued DRL training increased across blocks of testing in both groups. ANOVA confirmed these observations; a main within-group effect for Block yielded an $F(11, 198) = 10.332$, $p < .001$. No interaction between linear trends associated with the two groups was observed, indicating that the rate of increase in number of reinforcers obtained across blocks did not differ between groups.

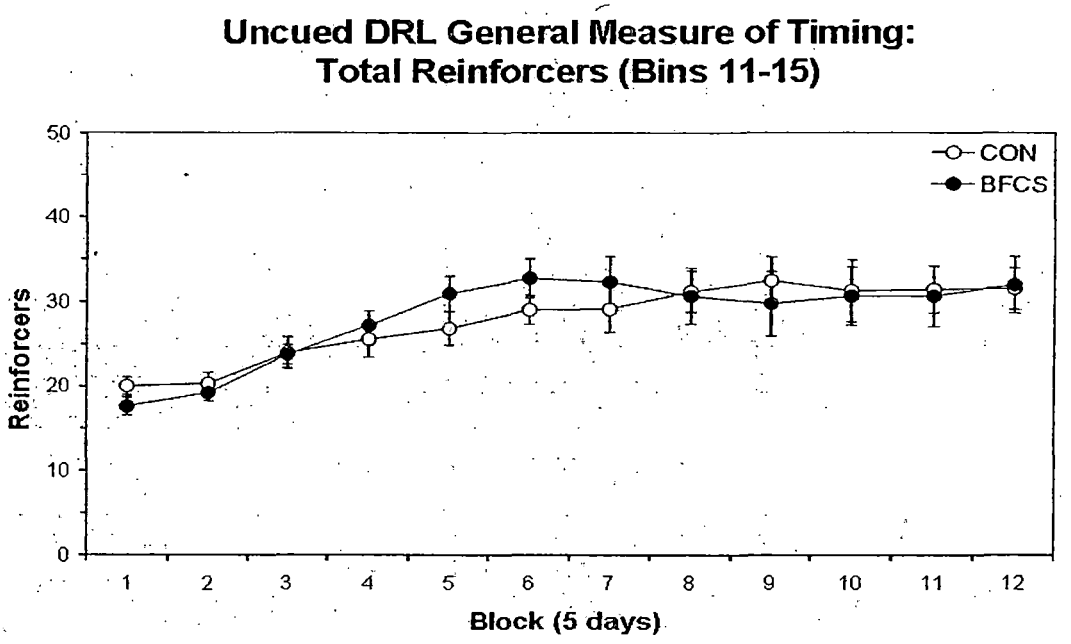


Figure 5. Mean (+/- SEM) Number of Total Reinforced Responses across the 10 s Reinforcement Window (i.e., Responses in Bins 11-15) for the Basal Forebrain Cholinergic System (BFCS) and Control (CON) Groups across Blocks (5 Days per Block) in the Uncued Differential Reinforcement of Low Rate Responding (DRL) Task. Groups did not differ in the number of total reinforced responses across the 10 s reinforcement window (i.e., bins 11-15) across blocks of testing. These data show that BFCS lesions do not impair general timing ability in the uncued DRL task.

No significant between-group differences in number of reinforcers obtained between the BFCS lesion group and the control group, nor any interaction between Group and Block were observed on this measure.

Precisely Timed (Bin 11) Responses

The mean number of bin 11 responses emitted by the BFCS lesion group and the control group during uncued DRL 20 s LH 10 s training are shown in Figure 6. The number of responses emitted in bin 11 during uncued DRL training increased across blocks of testing in both groups. ANOVA confirmed these observations; a main within-group effect for Block yielded an $F(11, 198) = 12.469$, $p < .001$. No interaction between linear trends associated with the two groups was observed, indicating that the rate of increase in number of reinforced bin 11 responses across blocks did not differ between groups.

No significant between-group differences in number of responses emitted in bin 11 between the BFCS lesion group and the control group, nor any interaction between Group and Block were observed on this measure.

Relatively Well-timed (Bins 12-15) Responses

The mean number of responses emitted in bins 12-15 by the BFCS lesion group and the control group during uncued DRL 20 s LH 10 s training are shown in Figure 7. The

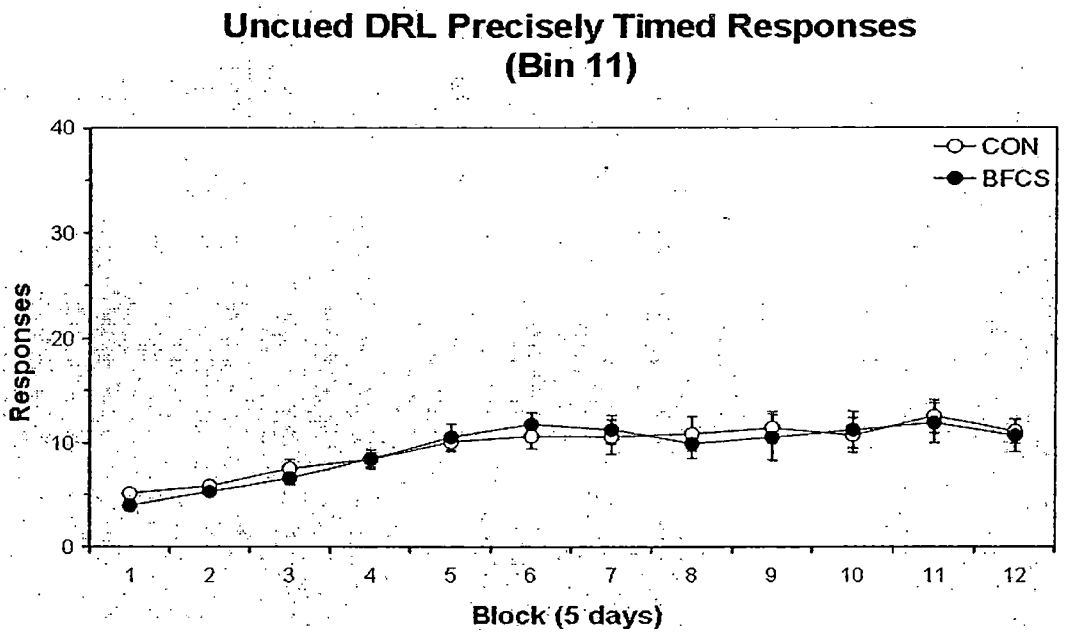


Figure 6. Mean (+/- SEM) Number of Reinforced Responses during the First 2 s of the Reinforcement Window (i.e., Responses in Bin 11) for the Basal Forebrain Cholinergic System (BFCS) and Control (CON) Groups across Blocks (5 Days per Block) in the Uncued Differential Reinforcement of Low Rate Responding (DRL) Task. Groups did not differ in the number of reinforced responses during the first 2 s of the reinforcement window (i.e., bin 11) across blocks of testing. These data show that BFCS lesions do not impair the ability to precisely time responses in the uncued DRL task.

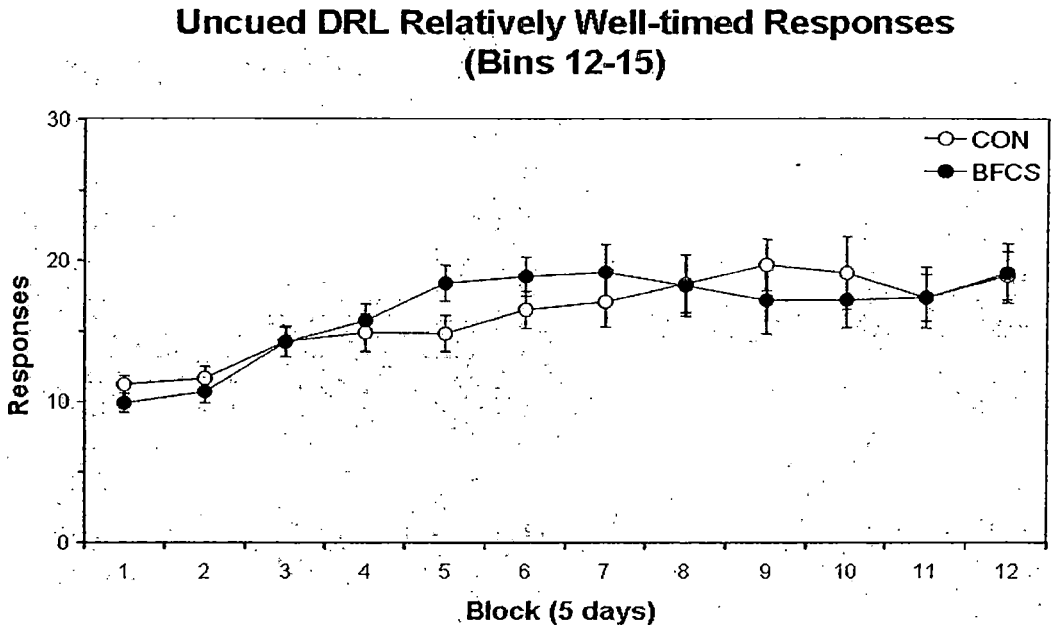


Figure 7. Mean (+/- SEM) Number of Reinforced Responses during the Latter 8 s of the Reinforcement Window (i.e., Responses in Bins 12-15) for the Basal Forebrain Cholinergic System (BFCS) and Control (CON) Groups across Blocks (5 Days per Block) in the Uncued Differential Reinforcement of Low Rate Responding (DRL) Task. Groups did not differ in the number of reinforced responses during the latter portion of the reinforcement window (i.e., bins 12-15) across blocks of testing. These data show that BFCS lesions do not impair the ability to relatively time responses in the uncued DRL task.

number of responses emitted in bins 12-15 during uncued DRL training increased across blocks of testing in both groups. ANOVA confirmed these observations; a main within-group effect for Block yielded an $F(11, 198) = 8.358$, $p < .001$. No interaction between linear trends associated with the two groups was observed, indicating that the rate of increase in number of reinforced responses in bins 12-15 across blocks did not differ between groups.

No significant between-group differences in number of responses emitted in bins 12-15 between the BFCS lesion group and the control group, nor any interaction between Group and Block were observed on this measure.

Efficiency

The mean efficiency scores obtained by the BFCS lesion group and the control group during uncued DRL 20 s LH 10 s training are shown in Figure 8. The efficiency scores obtained during uncued DRL training increased across blocks of testing in both groups. ANOVA confirmed these observations; a main within-group effect for Block yielded an $F(11, 198) = 33.039$, $p < .001$. No interaction between linear trends associated with the two groups was observed, indicating that rate of increase in efficiency across blocks did not differ between groups.

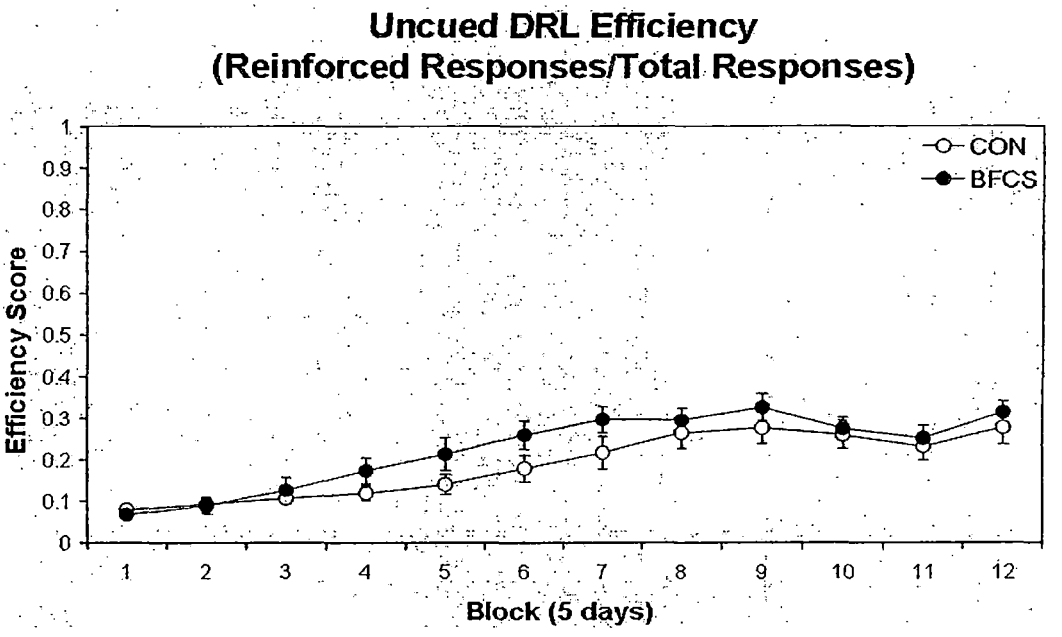


Figure 8. Mean (+/- SEM) Efficiency Scores for the Basal Forebrain Cholinergic System (BFCS) and Control (CON) Groups across Blocks (5 Days per Block) in the Uncued Differential Reinforcement of Low Rate Responding (DRL) Task. Efficiency scores did not differ between groups across blocks of testing. These data show that BFCS lesions do not disrupt overall efficiency performance in the uncued DRL task.

No significant between-group differences in efficiency scores obtained between the BFCS lesion group and the control group, nor any interaction between Group and Block were observed on this measure.

Cued Differential Reinforcement of Low Rate Responding

Performance across blocks of testing in the BFCS and control groups tested in the cued DRL task is shown in Figures 9 and 10. A detailed analysis of the constituent dependent variables in this task follows.

Total Responses

The mean number of total responses emitted by the BFCS lesion group and the control group during cued DRL 20 s LH 10 s training are shown in Figure 11. The number of total responses emitted during cued DRL training decreased across blocks of testing in both groups. ANOVA confirmed these observations; a main within-group effect for Block yielded an $F(11, 198) = 54.799$, $p < .001$. No interaction between linear trends associated with the two groups was observed, indicating that the rate of decrease in number of total response made across blocks did not differ between groups.

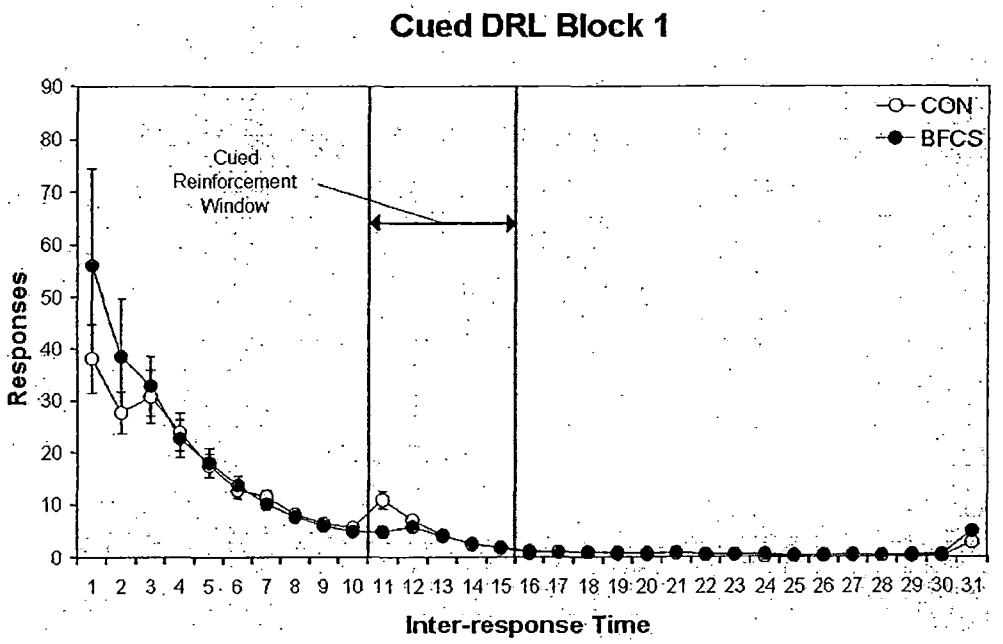


Figure 9. Mean (+/- SEM) Inter-response Time Distributions (2 s per Bin) for the Basal Forebrain Cholinergic System (BFCS) and Control (CON) Groups in the Cued Differential Reinforcement of Low Rate Responding (DRL) Task on Block 1 (Days 1-5). Note the rapid emergence of peak responding during the first reinforced interval (bin 11) in the control group as compared to the BFCS lesion group.

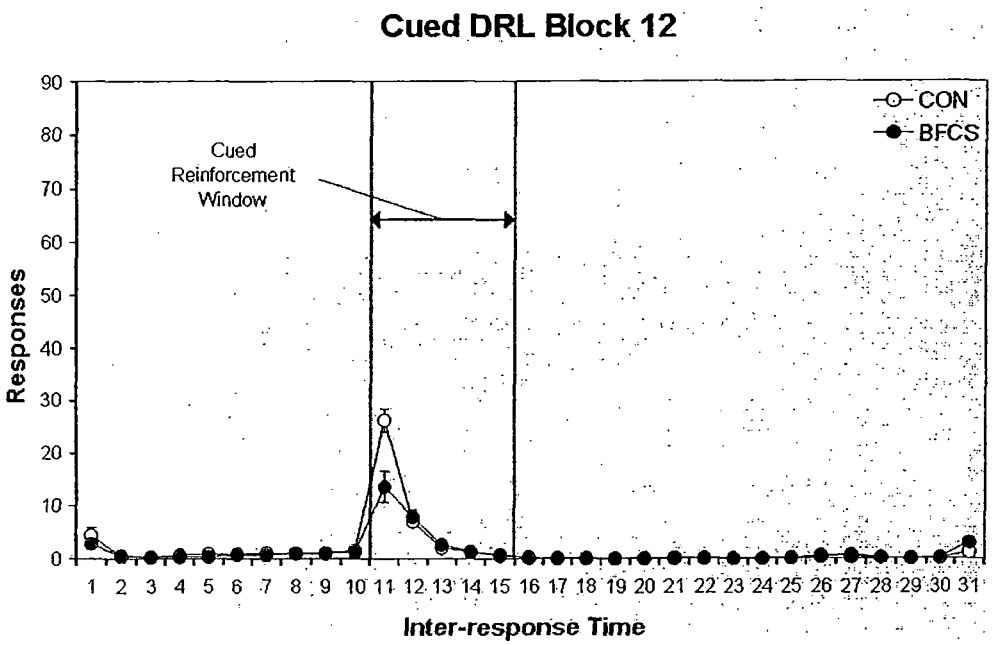


Figure 10. Mean (+/- SEM) Inter-response Time Distributions (2 s per Bin) for the Basal Forebrain Cholinergic System (BFCS) and Control (CON) Groups in the Cued Differential Reinforcement of Low Rate Responding (DRL) Task on Block 12 (Days 56-60). Note the greater number of responses during the first reinforced interval (bin 11) in the control group as compared to the BFCS lesion group across the 12 blocks (5 days per block) of testing. The control group is responding more quickly to the reinforcement cue than the BFCS lesion group.

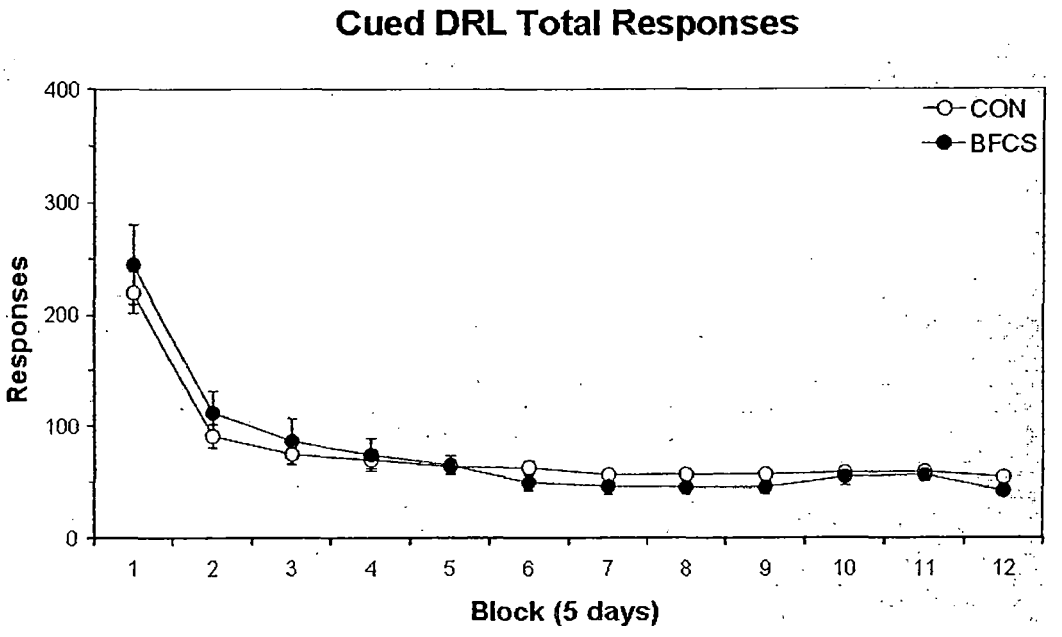


Figure 11. Mean (+/- SEM) Number of Total Responses across the Inter-response Time (IRT) Distribution (i.e., Responses in Bins 1-31) for the Basal Forebrain Cholinergic System (BFCS) and Control (CON) Groups across Blocks (5 Days per Block) in the Cued Differential Reinforcement of Low Rate Responding (DRL) Task. Groups did not differ in the number of total responses across the IRT distribution (i.e., bins 1-31) across blocks of testing. These data show that BFCS lesions do not impair the ability to inhibit perseverative responses in the cued DRL task.

No significant between-group differences in number total responses between the BFCS lesion group and the control group, nor any interaction between Group and Block were observed on this measure.

Perseverative (Bin 1) Responses

The mean number of bin 1 responses emitted by the BFCS lesion group and the control group during cued DRL 20 s LH 10 s training are shown in Figure 12. The number of responses emitted in bin 1 during cued DRL training decreased across blocks of testing in both groups. ANOVA confirmed these observations; a main within-group effect for Block yielded an $F(11, 198) = 15.75$, $p < .001$. No interaction between linear trends associated with the two groups was observed, indicating that rate of decrease in the number of responses emitted in bin 1 across blocks did not differ between groups.

No significant between-group differences in number of responses emitted in bin 1 between the BFCS lesion group and the control group, nor any interaction between Group and Block were observed on this measure.

Total Reinforcers (Bins 11-15)

The mean number of total reinforcers obtained by the BFCS lesion group and the control group during cued DRL 20 s LH 10 s training are shown in Figure 13. The number of

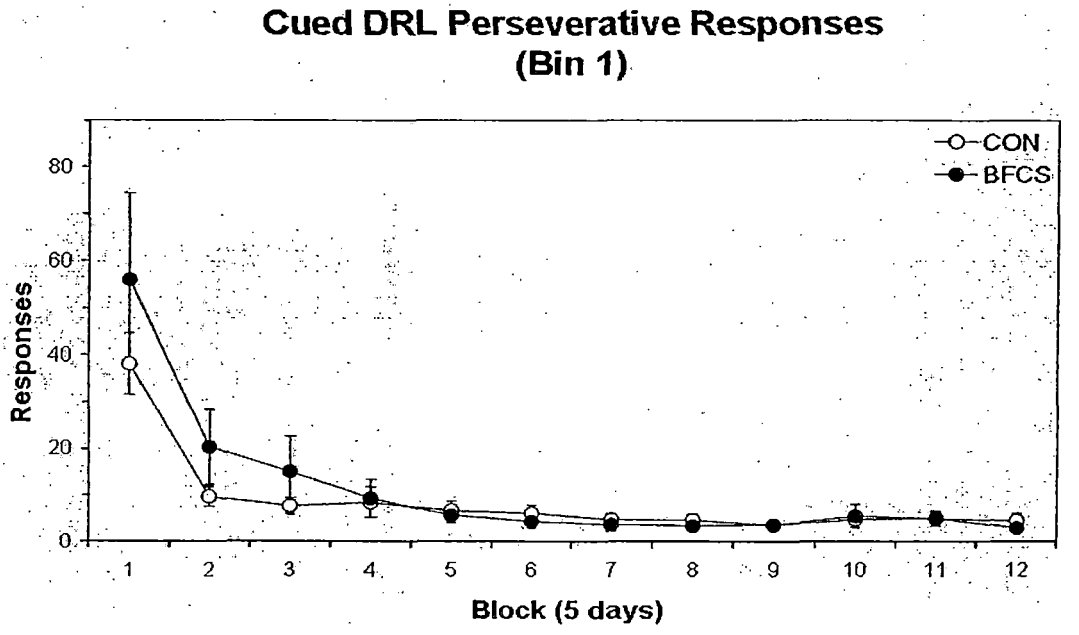


Figure 12. Mean (+/- SEM) Number of Short (0-2 s) Inter-response Time (IRT) Responses (i.e., Responses in Bin 1) for the Basal Forebrain Cholinergic System (BFCS) and Control (CON) Groups across Blocks (5 Days per Block) in the Cued Differential Reinforcement of Low Rate Responding (DRL) Task. Groups did not differ in the number of short IRT responses (i.e., bin 1) across blocks of testing. These data show that BFCS lesions do not impair the ability to inhibit perseverative responses in the cued DRL task.

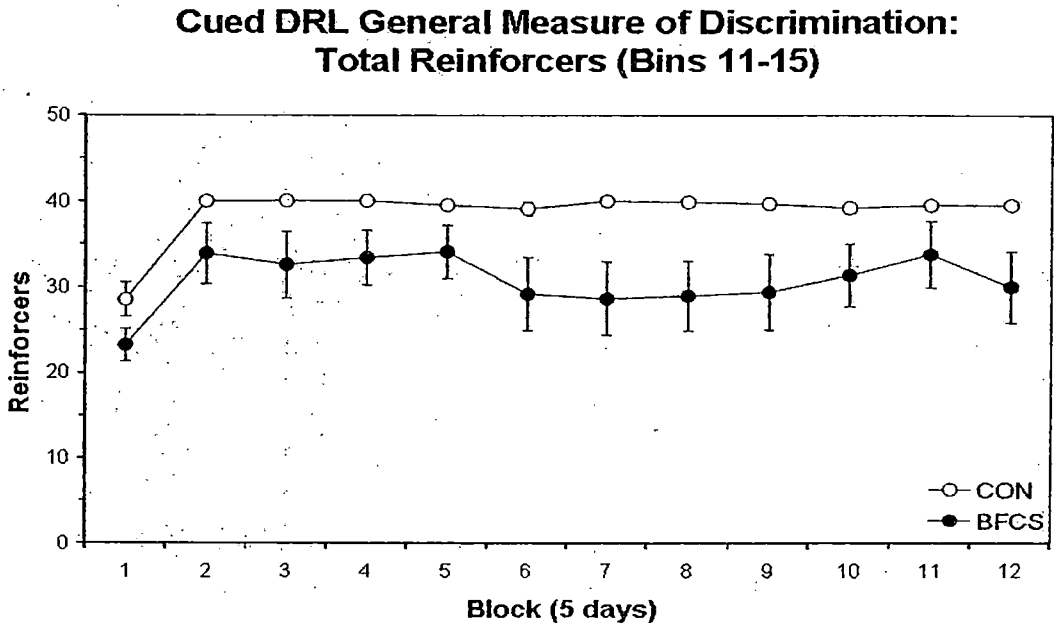


Figure 13. Mean (+/- SEM) Number of Total Reinforced Responses across the 10 s Reinforcement Cue Presentation (i.e., Responses in Bins 11-15) for the Basal Forebrain Cholinergic System (BFCS) and Control (CON) Groups across Blocks (5 Days per Block) in the Cued Differential Reinforcement of Low Rate Responding (DRL) Task. BFCS animals made fewer total reinforced responses overall across the 10 s reinforcement cue presentation (i.e., bins 11-15) across blocks of testing than the control group (main between-group effect, $p = .027$).

total reinforcers obtained during cued DRL training increased across blocks of testing in both groups. ANOVA confirmed these observations; a main within-group effect for Block yielded an $F(11, 198) = 9.694$, $p < .001$. No interaction between linear trends associated with the two groups was observed, indicating that the rate of increase in number of reinforcers obtained across blocks did not differ between groups.

Although the rate of increase in total number of reinforcements earned was not different in the two groups, the BFCS lesion group obtained fewer total reinforcers across testing blocks than controls. ANOVA confirmed these observations; a main between-group effect for Group yielded an $F(1, 18) = 5.796$, $p = .027$.

No interaction between Group and Block were observed on this measure.

Precisely Discriminated (Bin 11) Responses

The mean number of bin 11 responses emitted by the BFCS lesion group and the control group during cued DRL 20 s LH 10 s training are shown in Figure 14. The number of responses emitted in bin 11 during cued DRL training increased across blocks of testing in both groups. ANOVA confirmed these observations; a main within-group effect for Block yielded an $F(11, 198) = 12.701$, $p < .001$. No

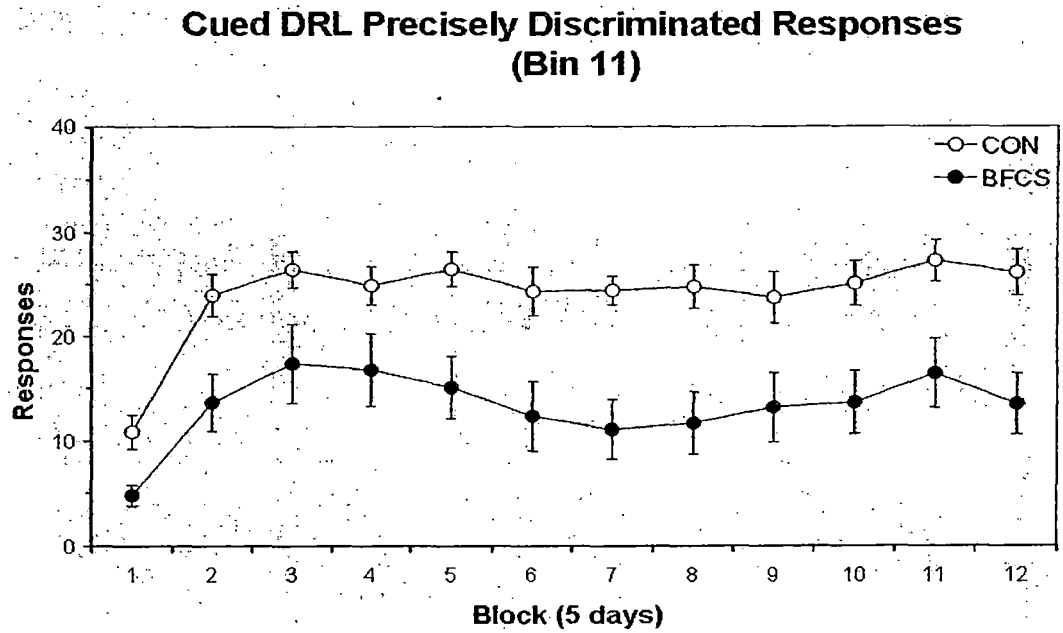


Figure 14. Mean (+/- SEM) Number of Reinforced Responses during the First 2 s of the Reinforcement Cue Presentation (i.e., Responses in Bin 11) for the Basal Forebrain Cholinergic System (BFCS) and Control (CON) Groups across Blocks (5 Days per Block) in the Cued Differential Reinforcement of Low Rate Responding (DRL) Task. BFCS animals made fewer reinforced responses during the first 2 s of the reinforcement cue presentation (i.e., bin 11) across blocks of testing than the control group (main between-group effect, $p = 0.002$).

interaction between linear trends associated with the two groups was observed, indicating that the rate of increase in number of reinforced bin 11 responses across blocks did not differ between groups.

Although the rate of increase in responses emitted in bin 11 was not different in the two groups, the BFCS lesion group made fewer responses in bin 11 across testing blocks than did the control group. ANOVA confirmed these observations; a main between-group effect for Group yielded an $F(1, 18) = 12.408$, $p = .002$.

No interaction between Group and Block was observed on this measure.

Relatively Well-discriminated (Bins 12-15) Responses

The mean number of responses emitted in bins 12-15 by the BFCS lesion group and the control group during cued DRL 20 s LH 10 s training are shown in Figure 15. The number of responses emitted in bins 12-15 during cued DRL training decreased across blocks of testing in both groups. ANOVA confirmed these observations; a main within-group effect for Block yielded an $F(11, 198) = 2.223$, $p = .015$. No interaction between linear trends associated with the two groups was observed, indicating that the rate of decrease in number of reinforced

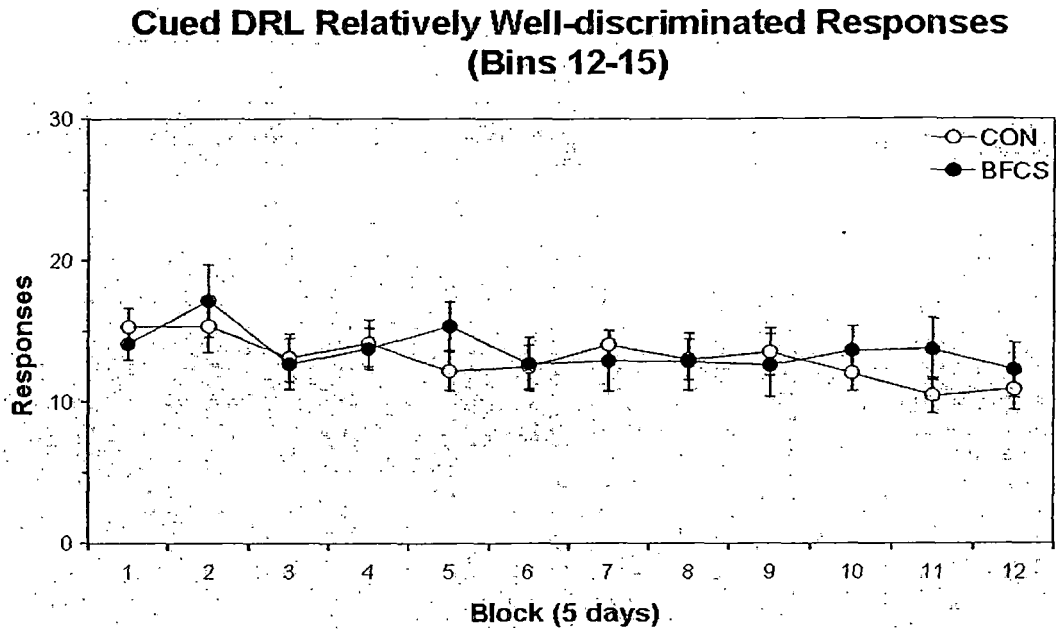


Figure 15. Mean (+/- SEM) Number of Reinforced Responses during the Latter 8 s of the Reinforcement Cue Presentation (i.e., Responses in Bins 12-15) for the Basal Forebrain Cholinergic System (BFCS) and Control (CON) Groups across Blocks (5 Days per Block) in the Cued Differential Reinforcement of Low Rate Responding (DRL) Task. Groups did not differ in the number of reinforced responses during the latter portion of the reinforcement cue presentation (i.e., bins 12-15) across blocks of testing. These data show that BFCS lesions do not impair the ability to relatively discriminate responses in the cued DRL task.

responses in bins 12-15 across blocks did not differ between groups.

No significant between-group differences in number of responses emitted in bins 12-15 between the BFCS lesion group and the control group, nor any interaction between Group and Block were observed on this measure.

Efficiency

The mean efficiency scores obtained by the BFCS lesion group and the control group during cued DRL 20 s LH 10 s training are shown in Figure 16. The efficiency scores obtained during cued DRL training increased across blocks of testing in both groups. ANOVA confirmed these observations; a main within-group effect for Block yielded an $F(11, 198) = 58.077$, $p < .001$. No interaction between linear trends associated with the two groups was observed, indicating that rate of increase in efficiency across blocks did not differ between groups.

No significant between-group difference in efficiency scores obtained between the BFCS lesion group and the control group, nor any interaction between Group and Block were observed on this measure.

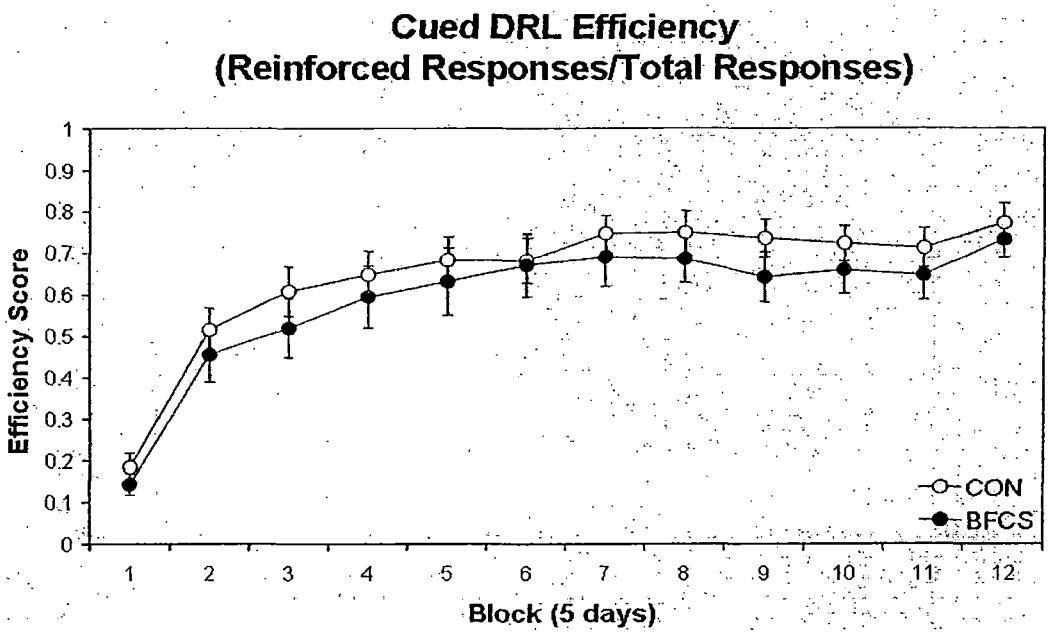
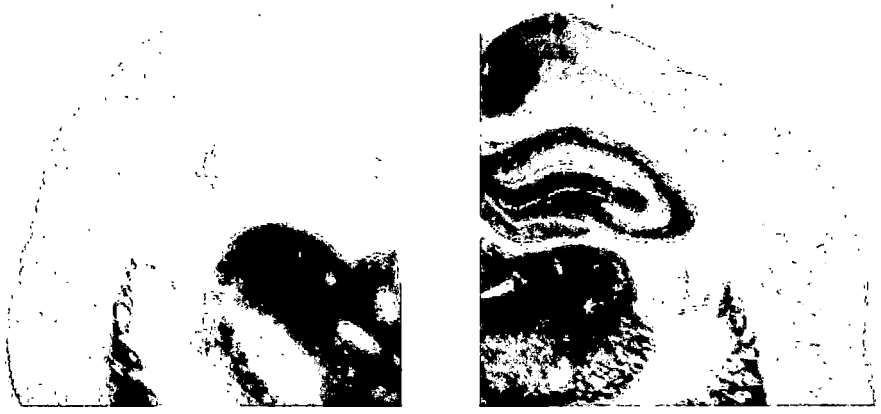


Figure 16. Mean (+/- SEM) Efficiency Scores for the Basal Forebrain Cholinergic System (BFCS) and Control (CON) Groups across Blocks (5 Days per Block) in the Cued Differential Reinforcement of Low Rate Responding (DRL) Task. Efficiency scores did not differ between groups across blocks of testing. These data show that BFCS lesions do not disrupt overall efficiency performance in the cued DRL task.

Histology

Figure 17 shows photomicrographs of coronal sections immunostained for AChE in a typical BFCS lesion rat (left side) and sham-operated control rat (right side). There is a dramatic reduction of AChE levels in the neocortex, the cingulate cortex, and the hippocampus of the BFCS lesion brain relative to the sham operated control. Thus, the observed impairments in the BFCS lesioned rats were most likely due to cholinergic hypofunction in these target areas.



BFCS CON

Figure 17. Acetylcholinesterase Stained Sections from a Typical Basal Forebrain Cholinergic System (BFCS) and Control (CON) Animal. Note the extensive loss of AChE-positive fiber staining in both the neocortex and hippocampus of the BFCS animal as compared to the control.

CHAPTER SEVEN

DISCUSSION

Uncued Differential Reinforcement of Low Rate Responding

In the uncued DRL experiment, results partially supported the hypothesis that SAP lesions of the BFCS would disrupt uncued DRL acquisition and performance by causing perseveration (i.e., increased responding with short inter-response intervals). Rats in the BFCS lesion group made more perseverative responses (i.e., bin 1 responses and total responses) than the control group early in training; later in training, however, the BFCS lesion group unexpectedly made fewer perseverative responses than the control group.

Although the BFCS lesion group initially emitted more perseverative responses than the control group, results did not support the hypothesis that this observed perseveration effect in the BFCS lesion group would correspondingly impair uncued DRL efficiency performance. Instead, rats in the BFCS lesion group performed as efficiently as controls across blocks of testing in the uncued DRL task. The extent of influence of the observed differences in bin 1 responding exerted on efficiency

scores appears to have been diluted as a function of similar levels of responding in all other bins (i.e., no significant between-group effect on total number of responses, responses in bin 11, bins 12-15, or bins 11-15). In other words, the compromise in efficiency caused by high level bin 1 responding was washed out by comparable levels of responding in all other bins.

Results supported the hypothesis that SAP lesions of the BFCS would not impair timing ability in the uncued DRL task. Rats in the BFCS lesion group performed as well as controls across blocks of testing on all timing measures (i.e., bin 11 responses, responses in bins 12-15, and total responses across the 10 s duration of the reinforced interval in bins 11-15).

In the following discussion section, a proposed explanation of the initial perseveration impairment in the uncued DRL task based on the argument that BFCS lesions disrupt the ability to continuously attend to the temporal processing or response inhibition requirements found in the uncued DRL task will be discussed. Additionally, the unanticipated finding that the BFCS lesion group eventually inhibited perseverative responding even more than controls in the uncued DRL task is discussed.

In the uncued DRL task, the ability to inhibit perseverative responses (in terms of the number of premature nonreinforced responses and in terms of total responses) was initially disrupted in the BFCS lesion group. This failure to inhibit responding in the uncued DRL task is consistent with previous research findings in rats with less selective lesions of the medial prefrontal cortex (Nalwa & Rao, 1985, 2001), medial septum (Ellen et al., 1964), and hippocampus (Braggio & Ellen, 1976; Clark & Isaacson) tested in an uncued DRL paradigm. Lesions of each of these structures typically impair uncued DRL performance by causing perseveration (i.e., an increase in responding at short inter-response intervals). Similarly, the observed results in the current experiment are consistent with the finding that systemic administration of the centrally acting anticholinergic drug scopolamine interferes with uncued DRL performance (Kelsey & Grossman, 1975; Meyer, Severson, & Thompson, 1976; Soffie & Lejeune, 1992) by causing an increase in the number of premature nonreinforced responses (i.e., perseveration).

The perseveration observed in the BFCS lesion group tested in the uncued DRL task in the current study may result from a lesion-induced disruption in the ability to continuously attend to the temporal processing or response

inhibition requirements of the uncued DRL task. Therefore, the perseveration impairment may be secondary to an underlying deficit in continuous attention. This interpretation is supported by the findings that continuous performance tests reveal impairments in patients with AD (Sunderland et al., 1989).

In the uncued task, the ability to inhibit perseverative responding ultimately recovers in the BFCS lesion group. This similar behavioral recovery has been reported in rats with SAP lesions of the NBM (Butt et al., 2002) and may suggest a role of other neuromodulatory systems or surviving cholinergic cells in compensating for the acetylcholine (ACh) loss in the BFCS lesioned animals in the current experiment. Indeed, the BFCS lesion group fully recovers the ability to inhibit perseveration, ultimately making significantly fewer perseverative responses than controls. This unexpected finding may reflect a secondary impairment of contextual conditioning in the BFCS lesion group, rather than reflecting superior performance in the BFCS lesion group as might first be assumed. One interpretation of these findings is based on the argument that an intact hippocampus is necessary for normal contextual conditioning (Rudy & O'Reilly, 1999). In the uncued DRL task, the animal is reinforced for

responding in the context of the operant chamber without any explicit external cues for reinforcement. Accordingly, the context would be expected to acquire some ability to control behavior. In this instance, the animal would be expected to respond on occasion simply as a function of such contextual conditioning, in addition to making more specifically timed responses matching the DRL schedule. Such contextually cued responding may appear as perseverative responding in the uncued DRL task. The BFCS lesion group, which has compromised hippocampal function as a result of damage to the cholinergic projections of the MS to the hippocampus, may not show normal contextual conditioning and as such may ultimately respond less than their control counterparts that do acquire contextual conditioning. Consistent with this argument, ACh levels have been found to increase in the hippocampus in normal rats both during acquisition of contextual fear conditioning and during subsequent re-exposure to the training context (Nail-Boucherie, Dourmap, Jaffard, & Costentin, 2000).

In the uncued DRL task, timing (i.e., responses in bin 11, responses in bins 12-15, and total responses in bins 11-15) was unimpaired in the BFCS lesion group. This sparing of timing in the uncued DRL task following SAP

lesions of the BFCS is consistent with previous findings in rats with less selective lesions of the frontal cortex, hippocampus, and septum tested in an uncued DRL paradigm. With respect to frontal cortical lesions, Nalwa and Rao (1985, 2001) have reported no timing deficit in rats with aspiration lesions of the medial prefrontal cortex tested in an uncued DRL task. Similarly, other researchers using the uncued DRL task have found that electrolytic lesions of the hippocampus in rats do not impair timing ability (Ellen et al., 1964). Equally, this sparing of timing ability is consistent with previous findings where rats with electrolytic lesions of the medial or lateral septal area developed a clear timing curve in an uncued DRL task (Brookes et al., 1983).

Cued Differential Reinforcement of Low Rate Responding

In the cued DRL experiment, results partially supported the hypothesis that SAP lesions of the BFCS would spare cued DRL acquisition and performance. Rats in the BFCS lesion group performed as well as the control group in inhibiting perseverative responses (i.e., bin 1 responses and total responses) and performed as

efficiently as controls across blocks of testing in the cued DRL task.

The ability to inhibit perseverative responses (i.e., premature nonreinforced responses and total responses) was spared in the BFCS lesion group. This sparing of response inhibition in the cued DRL task is consistent with previous findings in rats with less selective lesions of the frontal cortex, hippocampus, and septum tested in a cued DRL paradigm. Numan et al. (1975) found that rats with aspiration lesions to the medial prefrontal cortex were able to withhold responding during nonreinforced intervals, and did not show perseveration in a cued DRL task. Likewise, other studies have reported that electrolytic or aspiration lesions of the hippocampus of rats do not cause perseveration in cued DRL performance (Braggio & Ellen, 1976; Pellegrino & Clapp, 1971; Rickert et al., 1973). Similarly, the observed sparing of the ability to inhibit perseverative responding is consistent with previous findings where rats with electrolytic lesions to the septum were able to perform as well as controls in a cued DRL task (Braggio & Ellen, 1976; Ellen & Butter, 1969).

Results unexpectedly showed that despite the absence of perseverative responding and the absence of an

overall impairment in efficiency performance of the BFCS lesion group was disrupted in the cued DRL task. Specifically, discrimination of the reinforcement cue was poorer in the BFCS lesion group compared to controls. In particular, the BFCS lesion group made fewer responses in bin 11 (i.e., the first 2 s of the reinforcement cue presentation) and fewer reinforced responses overall (i.e., bins 11-15). However, further analysis revealed that the BFCS lesion and control groups did not differ in number of reinforced responses during the latter part of the reinforcement cue presentation (i.e., bins 12-15).

Together, this data suggest that vigilance or sustained attention to the reinforcement cue was impaired in the BFCS lesion group. Specifically, reaction time was slower in the BFCS lesion group compared to controls where this slower reaction time might reflect impaired vigilance. This argument is consistent with research on AD patients showing slowed reaction time in responding to both predictable and unpredictable external cues (Muller, Richter, Weisbrod, & Klingberg, 1991; Sano, Rosen, Stern, Rosen, & Mayeux, 1995).

Others have similarly argued that SAP lesions of the NBM result in a loss of vigilance, exclusively to brief and unpredictable visual stimuli (McGaughy et al., 1996).

However, in the cued version of the current DRL task, rats with SAP lesions of the BFCS were impaired in the ability to detect visual cues that were neither brief (cue light duration up to 10 s) or unpredictable (cues occurred every 20s from the most recent response). The more profound vigilance impairment observed in the cued DRL task in the current experiment may attributable to the more extensive damage to the BFCS caused by combined SAP lesions of the NBM and MS/VDB together. Others have shown that combined BFCS lesions using SAP can cause severe impairments where separate NBM or MS/VDB lesions may produce modest deficits or may fail to reveal any behavioral impairments at all (Dornan et al., 1997; Pizzo et al., 2002).

Summary

To summarize, in the uncued DRL task, BFCS lesions caused a transient perseveration without affecting the ability to time. The observed perseveration was consistent with the hypothesis and was interpreted as reflecting an impairment in continuous attention. The subsequent recovery of response inhibition beyond control levels was unanticipated. An argument based on the idea that hippocampus dependent contextual conditioning may have kept control responding elevated, while the BFCS

lesion group may have failed to undergo contextual conditioning as a result of MS lesion-induced cholinergic denervation of the hippocampus was presented.

In the cued DRL task, BFCS lesions did not cause perseveration, as was predicted. Surprisingly, discrimination of the reinforcement cue was impaired in the BFCS lesion group. This impairment was characterized as a slowed reaction time in responding to the reinforcement cue. This reaction time impairment was argued to reflect an underlying vigilance decrement.

A common underlying impairment in continuous attention or vigilance may affect performance in the uncued and cued DRL tasks differently. Although the BFCS lesion group initially failed to inhibit responding in the uncued DRL task, no such perseveration effect was observed in the cued version of the task. This difference may reflect the fact that in the uncued DRL task, continuous attention to timing and response inhibition are required, whereas focused attention to these requirements is not necessary in the cued DRL task. In the uncued task, a failure of continuous attention would therefore account for the perseveration observed on several trials, despite the ability to time responses normally on other trials. When rats are attending to the uncued DRL task

requirements, performance is normal. However, any disruption in continuous attention would impair performance by causing premature or untimed responses. In contrast, in the cued DRL task, where continuous attention to timing and response inhibition are not explicitly required and animals need only to attend to the reinforcement cue, impaired vigilance to the reinforcement cue would result in slower reaction times, and a resulting decrease in overall reinforcement.

Although behavioral recovery was observed in the BFCS lesion group tested in the uncued DRL task, no such recovery was observed in the BFCS lesion group tested in the cued DRL task. An argument based on task difficulty does not appear to apply here; most researchers would agree that the uncued DRL task is much more difficult than the cued DRL task. Instead, an external cue versus internal cue attention interpretation appears to fit with the current data from the cued and uncued DRL tasks. That is, the BFCS lesion group appears to be impaired in quickly responding to external (but not internal) cues. In the cued DRL task, the BFCS lesion group responded normally at longer reaction times (i.e., 2-10 s, bins 12-15), but was impaired at shorter reaction times (i.e., 0-2 s, bin 11 responses). In contrast, in the uncued DRL

task, the BFCS lesion group did not differ from the control group on any reaction time measure (i.e., bins 11-15). Results suggest that vigilance and attention to external cues is impaired following SAP lesions of the BFCS despite continued practice in the cued DRL task, whereas continuous attention to internally produced cues recovers with extended practice in the uncued DRL task.

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